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THE RELATION OF FLOWERING AND CAMBIAL ACTIVITY

OBSERVATIONS ON VASCULAR DIFFERENTIATION AND DRY-WEIGHT CHANGES IN THE CATKINS OF SOME EARLY FLOWERING CATKIN-BEARING DICOTYLEDONS

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INTRODUCTION

CAMBIAL activity in the woody Dicotyledon has been shown to commence in spring in connection with the developing foliar organs and to proceed from thence down to the older branches and trunk.

The literature giving rise to these conclusions is summarised and discussed in a paper by Priestley[10] to which an extensive bibliography is attached. In the paper cited reference is made to an apparent exception to the above rule, in the observations of Chalk[1] on the common ash (*Fraxinus excelsior* L.), and it is suggested that in this species cambial activity may begin first in connection with the opening inflorescence buds and proceed basipetally from thence before activity commences in connection with the later opening vegetative buds. This suggestion, which has been found to be correct, has directed attention to the relation of floral development in general to cambial activity.

Reference to the literature shows that little information is available regarding the differentiation of vascular tissue in connection with floral organs. Coster[3] records observations on tropical plants showing that renewed cambial activity is found before new leaves appear, in *Prunus puddum* and *Magnolia obovata*, both of which flower before the appearance of new leaves. Further knowledge on the subject is obviously of some importance not only in connection with the inception of cambial activity in trees which flower before

the leaves appear, but also in connection with the closely associated problem of the translocation of food to the growing organs.

An examination of this aspect of cambial activity has therefore been commenced, and the following pages contain a report of the changes observed in the vascular structure of the stalk of the male inflorescences of four catkin-bearing trees. Observations were made during the dormant winter period and the period of opening of the catkins in the spring. The species examined were poplar (*Populus serotina* Hartig.), willow (*Salix caprea* L.), hazel (*Corylus avellana* L.) and alder (*Alnus glutinosa* Gaert.). The first two species have catkins contained in buds during the winter from which they emerge when growth commences in spring, whilst the last two have their catkins exposed throughout the winter.

In connection with the problem of translocation the changes in fresh and dry weight and ash content were followed at various stages of development, and these have been correlated with the observed vascular differentiation in the catkin stalk.

The observations are placed on record in the following pages and a brief discussion of the results follows.

DESCRIPTION OF MATERIAL AND METHODS

Populus serotina. One-year-old flowering twigs were collected from a tree about 60 ft. high growing in the grounds of Weetwood Hall, Leeds. All collections were made from two branches about 15 ft. above ground level. The flowering twigs during the winter have from three to five large lateral inflorescence buds and sometimes toward the base of the twig one or two smaller buds. The whole of the buds are arranged in a 2/5 phyllotaxis, and at the apex of the twig is a vegetative bud which continues the elongation growth of the twig in spring. Each inflorescence bud contains a single male catkin on a short stalk bearing two or three very small scale leaves. The catkins appear from the buds in spring before the vegetative buds begin growth, and about a month is occupied in the emergence from the bud and the completion of the growth of the catkin.

Salix caprea. Flowering twigs were collected from two similar bushes, one growing by the roadside at Knaresborough, Yorks. and the other in a similar position at Adel, Leeds. The twigs have a false terminal vegetative bud and a number of lateral buds, most of which are inflorescence buds and contain a single catkin, but the first one or two below the apex are often vegetative. The catkins have very short stalks and bear a few basal scale-like leaves. The catkins emerge

from the buds in spring and complete their flowering before the leaves appear from the vegetative buds.

Corylus avellana. Twigs bearing male catkins were collected from two bushes forming part of a hedge to the University athletics grounds in Leeds. The catkins are borne exposed during the winter on short lateral branches, sometimes singly and sometimes two to four on a branch. Elongation of the catkins occurs in spring before the leaves appear.

Alnus glutinosa. Flowering twigs were collected from branches about 8 ft. above ground level, of a tree about 40 ft. high growing on the bank of a stream at "Meanwoodside," Leeds. The catkins are exposed throughout the winter in groups of two to four, which are borne at the apex of the one-year-old twig. The catkins elongate in spring before the leaves appear.

In each case (except *Salix* which was collected less frequently) collections were made during the dormant winter period at intervals of two to four weeks and at more frequent intervals during the active flowering period. The twigs with catkins attached were pickled in 90 per cent. methylated alcohol. Transverse and longitudinal sections of the catkin stalks were obtained and stained with safranin and light green. Sections of the catkin-bearing twig were also made when required and stained similarly.

Dry-weight determinations were made on the dates shown in Tables I–IV. Catkins of as nearly uniform size as possible were removed, and after previous weighing were dried in a steam oven. For *Corylus* and *Alnus* thirty catkins were used for each set of figures, whilst in the cases of *Populus* and *Salix* figures are for twenty catkins. For the first seven collections of *Populus* it was necessary to remove the bud scales in order to obtain the catkins alone, this was also necessary for the first weighing of *Salix*. The ash content was obtained by burning the dried catkins in porcelain crucibles.

In the cases of *Corylus* and *Alnus* the catkins have a small amount of chlorophyll, and as they are exposed throughout the series of weighings it was thought advisable to obtain additional readings for catkins not exposed to the light in order to obtain some idea of the influence on the dry weight of any photosynthetic activity which might occur. A number of twigs bearing catkins were therefore covered with closely woven black cloth, supported by copper wire to prevent it from touching the catkins, and fastened on to the twigs. The covered twigs were supported by strings attached to surrounding twigs. The *Corylus* twigs were covered on November 21st, 1931, and

Alnus on November 16th, 1931. In the following record of observations the term *differentiating wood* is used in describing all stages in the formation of the fully lignified wood elements devoid of protoplasmic contents from the cambial cell derivative. *Mature sieve tubes* are regarded as elements in which the sieve plates and companion cells have formed and the nuclei of the sieve tube have disappeared, but protoplasmic contents are still present. *Differentiating sieve tubes* include all stages in the formation of mature sieve tubes from the cambial cell derivative, in which the nuclei of the tube are still present.

RECORD OF OBSERVATIONS
Poplar (Populus serotina)

At the base of the axis of the inflorescence during winter the vascular tissue is arranged in a circle and is broken up by a number of large rays into between thirty and forty distinct bundles in transverse section. Each bundle contains a few small spiral tracheids separated from the phloem by a regular cambial layer of four to ten rows. The phloem, which is larger in volume than the wood, consists of small sieve tubes and a large proportion of parenchyma.

The catkin buds remained dormant until about March 31st, when they were beginning to swell and the bud scales were slightly displaced. On this date no differentiation of new vascular tissue could be detected. On April 8th the catkins had increased to about double their original size and the bud scales were widely displaced. In the catkin stalk the phloem and cambium were slightly swollen and new sieve tubes and wood elements were differentiating, but no lignification of the wood had taken place. Five days later two rows of lignified wood had been formed and a further row was differentiating. Differentiating sieve tubes were also present on this date. The new wood elements were spirally lignified and larger than those which had been present throughout the winter. Some of the elements formed later were pitted. The trace from the catkin enters the stele of the twig very gradually and it was possible to obtain a series of sections showing the entry of the trace and the ultimate union with the stele of the twig. This series showed that differentiation of wood and phloem continues in the trace until it has joined up with the stele of the twig, but below this no differentiation of wood occurs in connection with the catkin. Thus differentiation of wood in connection with the opening of the inflorescence in spring does not proceed basipetally down the twig to the main branches and trunk

from the opening inflorescences, as has been found to be the case in the common ash (*Fraxinus excelsior*), but stops short in the region where the inflorescence trace becomes connected with the stele of the twig. It was noted, however, that the phloem of the twig became swollen and new sieve tubes were differentiated below the point of entry of the traces from the catkins, and differentiating sieve tubes were also present in small numbers in the two-year-old twig. On May 9th all the catkins had fallen. Below the terminal bud three rows of differentiating wood and some differentiating sieve tubes were present. Thus after growth of the catkins is over growth commences in the terminal vegetative bud, and in connection with this the cambium becomes active in the production of both wood and phloem throughout the twigs.

The absolute ash content increases during the expansion of the catkins, but the ash content as a percentage of the dry weight shows a decrease from 8·94 in dormant catkins to 6·21 in expanded catkins.

TABLE I

Poplar (Populus serotina)

Dry-weight determinations. Each set of figures represents values for twenty male catkins

Date of collection	Fresh weight grm.	Dry weight grm.	Dry matter %
Feb. 10th	1·257	0·518	41·21
„ 24th	1·318	0·529	40·21
Mar. 17th	1·136	0·434	38·21
„ 31st	2·290	0·600	26·36
Apr. 8th	3·389	0·745	21·97
„ 13th	5·844	1·105	18·91
„ 23rd	13·043	2·210	16·90
„ 30th	14·734	1·973	13·39

Table I shows the changes in the fresh and dry weight of twenty male catkins during the period of the observations. If the dry weights in the first three samples are regarded as representing the dry weight during the dormant period and the dry weights in the last two as representing the dry weight toward the end of growth of the catkins, it will be seen that a significant gain in dry weight occurs during the expansion of the catkins of poplar in spring. Differentiation of new vascular tissue begins as the water content increases and, together with this, absolute dry weight starts to rise. The figures suggest a steady increase in dry weight accompanied by a more rapid increase in water content, and during the whole of this period it seems significant that differentiation of vascular tissue continues. Very little chlorophyll is present in the catkins, so that all gain in

dry weight must be derived from substances transferred into the catkins from the twig. The small increase in ash content of the catkins can only account for a very slight increase in dry weight, and it is clear that the greater part of the dry-weight increase must be due to transferred organic matter.

Willow (Salix caprea L.)

The wood in the dormant inflorescence axis consisted of a few small groups of small spiral tracheids separated by large rays. Between the wood and the phloem, which consisted of small sieve tubes and larger parenchyma cells, was a regular cambial layer of from three to eight rows of cells.

As in the case of *Populus*, when the catkins opened in spring new wood and phloem were formed in the catkin axis and the existing phloem became swollen. Here again activity in differentiation of wood did not spread down the twig, but differentiating sieve tubes were observed lower down the twig below the open catkins.

Weighings were only made of catkins in the dormant buds on February 28th and when opening of the catkins was complete and the stamens were exposed on April 12th. The figures for these weighings, which are given in Table II, suggest that a large increase in dry weight occurs together with the increase in water content as the male catkins grow and vascular differentiation occurs in spring. The change in ash content is only small, so that most of the increase in dry weight must be due to increase in organic matter transferred to the catkins from the twig, photosynthesis being negligible owing to the small amount of chlorophyll in the catkins.

TABLE II

Willow (Salix caprea)

Dry-weight and ash determinations. Each set of figures represents values for twenty male catkins

Date of collection	Fresh weight grm.	Dry weight grm.	Dry matter %	Ash grm.	Ash as % of dry matter
Feb. 28th	0·617	0·253	41·00	0·030	11·86
Apr. 12th	6·999	1·411	20·16	0·095	6·74

Hazel (Corylus avellana)

In the stalk of the dormant catkin during winter the wood consists of a circle of from six to twelve rows of small spirally thickened tracheids, broken radially by narrow rays. The phloem consists of small sieve tubes and a large proportion of parenchyma. Phloem

and xylem are separated by a layer of about four rows of un-differentiated cambial cells.

On February 23rd the catkins had begun to elongate, and on March 2nd many had attained their maximum size and had shed their pollen. The only change in the vascular structure of the catkin stalk noted as the catkins elongated was a swelling of the nuclei of the parenchymatous cells, and no new wood or phloem was differentiated.

Table III shows the changes in weight and ash content during the dormant winter season and during the period of elongation in spring. On October 13th the leaves had not yet fallen and the average dry weight is low compared with the average during the winter, which suggests that the catkins had not quite reached their maximum size before the dormant winter period. For the weighings made during the dormant period from December 8th to February 15th the dry weight varies only slightly in six sets of figures, the average dry weight being 1·524 grm. and the variation ±0·103 grm.

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TABLE III

Hazel (Corylus avellana)

Dry-weight and ash determinations. Each set of figures represents values for thirty catkins

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Date of collection		Fresh weight grm.	Dry weight grm.	Dry matter %	Ash grm.	Ash as % of dry matter
Oct. 13th	I	3·115	1·249	40·10	—	—
	II	2·915	1·130	38·76	—	—
	III	2·411	0·949	39·36	—	—
Dec. 8th	I	4·100	1·602	39·07⎱	0·156	4·84
	II	4·149	1·624	39·14⎰		
Jan. 11th		3·940	1·500	38·07	—	—
Feb. 1st	I	3·944	1·421	36·03⎱	0·177	6·22
	II	3·961	1·423	35·93⎰		
,, 15th		4·244	1·574	37·09	—	—
,, 23rd		4·984	1·602	32·14	0·096	5·99
Mar. 2nd	I	4·780	1·496	31·30	0·084	5·61
	II	5·332	1·668	31·28	0·100	5·99
	III	4·978	1·544	31·02	0·090	5·83
Mar. 2nd*	I	4·260	1·460	34·27	0·075	5·14
	II	4·915	1·740	35·40	0·090	5·17

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* Covered catkins.

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On March 2nd the catkins had expanded, care being taken to use only catkins which had not yet shed their pollen. For the three sets of figures for uncovered catkins on this date the average dry weight is 1·569 grm., so that there obviously is no significant difference between the dry weight of dormant catkins in winter and expanded catkins in spring. The percentage dry matter for the six weighings from December 8th to February 15th shows an average of 37·56

with a variation of $\pm 1 \cdot 63$. On March 2nd the percentage dry matter has fallen to an average of $31 \cdot 20$, and as the absolute dry weight has remained constant the water content shows a significant increase. The changes in ash content show no significant variation in uncovered catkins over the period of observation. The only difference shown by the covered catkins is a less pronounced decrease in percentage dry matter, which suggests that increase of water content is not quite so great in covered catkins as in exposed catkins. The figures do not indicate that photosynthetic activity plays any part in the opening of the catkins in spring, and no difference could be detected in the appearance of the covered and exposed catkins.

It seems clear that there are two significant differences between the changes occurring in the catkins of *Corylus*, and those of *Populus* and *Salix*, as they elongate in spring. First there is no change in dry weight, and secondly there is no differentiation of new vascular tissue. It is significant in connection with the second point that differentiation of the pollen grains is complete before the beginning of the winter rest, and it seems probable that all that occurs in spring in the hazel catkin is the elongation of the catkin due to swelling of the cells following intake of water and that no actual cell differentiation or division takes place.

Alder (Alnus glutinosa)

The vascular structure of the stalk of the dormant catkin of *Alnus* was similar to that of *Corylus*.

In this case the catkins were still apparently dormant on March 14th, but on April 4th they had elongated and many had shed their pollen. Here again there was no differentiation of new vascular tissue and the only change noted was the swelling of the nuclei in the parenchymatous cells.

Table IV shows the variations in fresh weight, dry weight, and ash content over the period of observation. On October 13th the leaves had not yet fallen and it is possible that growth of the catkins was not complete, so that the average dry weight on this date may be rather lower than that for dormant catkins. The seven weighings from November 16th to February 15th may be taken as representative figures for dormant catkins during winter. These figures show an average dry weight of $2 \cdot 417$ grm. with a variation of $\pm 0 \cdot 197$ grm. On April 4th the catkins had expanded and care had to be taken to select only catkins which still retained their pollen. The average dry weight on this date ($2 \cdot 364$ grm.) shows no significant variation from

the average for dormant catkins. Here again the figures indicate a slight increase in the percentage of water present in the catkins, but the increase is not so pronounced as in *Corylus*. No significant change in the ash content of the catkins is found throughout the series of observations. As in the case of *Corylus* no significant difference is shown between the figures for covered and exposed catkins.

TABLE IV

Alder (Alnus glutinosa)

Dry-weight and ash determinations. Each set of figures represents values for thirty catkins

Date of collection		Fresh weight grm.	Dry weight grm.	Dry matter %	Ash grm.	Ash as % of dry matter
Oct. 13th	I	5·553	2·323	41·83	—	—
	II	4·824	1·980	41·04	—	—
	III	5·308	2·203	41·50	—	—
Nov. 16th	I	5·523	2·220	40·20)	0·159	3·35
	II	5·995	2·534	42·27)		
Dec. 8th		5·973	2·608	43·66	0·082	3·14
Jan. 11th	I	6·055	2·510	41·45	—	—
	II	5·768	2·463	42·70	—	—
Feb. 15th	I	5·652	2·358	41·72	—	—
	II	5·387	2·224	41·28	—	—
Mar. 14th		5·747	2·262	39·36	0·066	2·48
Apr. 4th	I	6·362	2·452	38·54	0·072	2·93
	II	5·855	2·285	39·03	0·063	2·76
	III	6·258	2·356	37·65	0·070	2·97
Apr. 4th*	I	6·200	2·252	36·32	0·066	2·93
	II	6·144	2·228	36·26	0·054	2·42

* Covered catkins.

Here again the pollen grains were differentiated before the beginning of the winter rest, so that the state of development in the dormant catkins of *Alnus* during the winter is the same as that of the catkins of *Corylus*. The mature condition of the pollen grains in mid-winter in the catkin of *Alnus glutinosa* has already been recorded by Chamberlain [2] who found a similar condition to exist in *Corylus americana*.

DISCUSSION

The catkin-bearing species which flower before the leaves appear may be divided into two groups:

(1) Those in which the catkins are in buds at the beginning of spring activity, as exemplified by *Populus serotina* and *Salix caprea*.

(2) Those with catkins exposed at the beginning of spring activity, as in the cases of *Corylus avellana* and *Alnus glutinosa*.

It seems clear that the catkins in the second group are more fully differentiated at the beginning of winter than are those of the first group, and that in spring no further differentiation occurs and expansion of the catkin takes place as a result of intake of water which brings about the swelling of the existing cells.

No measure of respiration during the period under observation has been obtained, so that it is not possible to say definitely that no dry matter is gained from the twig, as this gain might be exactly balanced by loss due to respiration. It does not seem likely, however, in view of the absence of meristematic activity in the catkin, that any considerable loss due to respiration takes place. It seems probable, then, that in such catkins no significant amount of organic matter is withdrawn from the twig as the catkins expand in spring.

In *Populus* and *Salix* a definite increase in dry weight due to withdrawal of organic matter from the twigs occurs, and is accompanied by renewed differentiation of vascular tissue in the catkin and its trace into the twig.

Curtis (4, 5, 6) and Mason and Maskell (8, 9) have put forward evidence to show that organic matter is translocated in the phloem and most probably in the sieve tubes. Priestley, in a review of Mason and Maskell's work (11) and in a recent paper (10), has suggested that *differentiating* sieve tubes may be chiefly concerned in the movement of carbohydrates. Anatomical observations in Leeds on *Acer pseudoplatanus* (Hinton, unpublished thesis) and *Fraxinus excelsior* (Gill (7)) suggest such elements as the probable channels of movement of substances down from the leaves in summer. The facts presented in this paper seem significant in connection with these translocation problems. In *Corylus* and *Alnus* the dry weight remains constant and renewed differentiation of vascular tissue does not occur, whilst in the other species dry weight increases and new vascular tissue is formed. This at once suggests a correlation between differentiation of vascular tissue and movement of material upwards to the catkin from the twig. The translocation may take place in the differentiating wood or the differentiating sieve tubes. As differentiation of wood stops short soon after the trace of the catkin has joined up with the stele of the twig, and phloem differentiation occurs much lower down the twig, it seems possible that movement of organic solutes into the catkins takes place from regions of the twig for some distance below and that such movement occurs in the differentiating sieve tubes. Some movement of substances upwards may result from the absorption of organic solutes by the

differentiating wood elements, in the trace of the catkin, from the surrounding tissues of the twig, these being liberated into the differentiated wood above, in the axis of the catkin.

While the possibility of movement of solutes in the fully differentiated wood or phloem still remains, the recorded observations seem to point to the dependence of the growing organs on the renewed differentiation of vascular tissue for translocated food materials.

It is interesting in this connection to note that differentiation of wood and phloem continued in the catkin stalks of *Corylus* and *Alnus* during the period in which actual formation of the floral organs and all dry-weight increase would occur, before the dormant winter condition was attained.

In conclusion it is clear that unlike the case of *Fraxinus excelsior* already cited (10) the opening of the flowers in both the first and second group of catkin-bearing plants does not influence the inception of wood production in the twigs and older parts of the tree, and that its inception in these parts is associated with the beginning of growth of the vegetative buds. Slight phloem production is, however, found in the twigs in connection with the catkins of the second group, but such activity is short lived, terminating in the abscission of the catkins, and is soon masked by differentiation of phloem in connection with the terminal vegetative buds. Thus it is clear that no generalisation is yet possible as to the relation of flowering and cambial activity. It seems clear that there is a connection between cambial activity and the stage of differentiation which had been reached by the inflorescence before the beginning of renewed activity, but some other factor, which determines whether differentiation of wood shall, or shall not, proceed from the inflorescence down into the twigs and older parts of the tree is involved, and it is hoped to obtain further information on this subject by detailed work on other species.

SUMMARY

1. Renewed differentiation of wood and phloem occurs in the axis of the male catkins of *Populus serotina* and *Salix caprea* as the catkins expand in spring. At the same time phloem differentiation is found throughout the twigs below the expanding catkins, but wood production does not commence in the twig beyond the region at which the catkin trace unites with the stele of the twig.

2. The absolute dry weight increases during expansion of the catkins.

3. Most of the dry-weight increase is due to organic matter withdrawn from the twigs.

4. No renewed cambial activity occurs in the male catkins of *Corylus avellana* and *Alnus glutinosa*, and the absolute dry weight of the catkins as they expand shows no significant change.

5. The facts are discussed in relation to the translocation of substances to the expanding catkins and to the problem of the inception of cambial activity in trees.

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A PHYSIOLOGICAL INVESTIGATION OF THE EPHEMERAL FLOWERS OF *TURNERA ULMIFOLIA* L. VAR. *ELEGANS* URB.

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(With Plate I and 3 figures in the text)

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INTRODUCTION

FROM the physiological point of view the production of so-called ephemeral flowers appears to be a wasteful process. In such flowers the corolla persists for a very short time and then, independently of its success or failure in attracting pollinating insects, it dies and is discarded. Lepeschkin (3) has shown that the short life of the ephemeral corolla of *Cichorium Intybus* is due to the particularly great instability of the principal compounds of its protoplasm. The fading of these flowers was found to have no connection with fertilisation nor to be produced by lack of humidity.

Turnera ulmifolia L. var. *elegans* Urb. (referred to henceforth, for the sake of brevity, as *Turnera elegans,* the name which is commonly used), affords very suitable material for the study of ephemeral flowers, and the following is an account of an investigation of these flowers with particular reference to the effect of nocturnal illumination on the opening of the buds and to the movements of water and dissolved substances into and out of the flowers.

This plant, which is a woody perennial herb, is a native of Brazil,

but is commonly grown in Colombo gardens. It flowers profusely throughout the whole year, in Colombo at any rate. The ephemeral nature of the flowers is very marked. The flowers open about 2 hours after dawn and they close and become withered 3–4 hours later. Each flower is about 5 cm. in diameter and, as the flowers are produced in large numbers, the change in the appearance of the plants during the morning hours is very striking. The opening and closing movements are comparatively rapid, and these changes take place in all the flowers at about the same time.

The general appearance of the tip of a flowering shoot is shown in Text-fig. 1. Normally, only one flower opens at a time on each

Text-fig. 1. Tip of flowering shoot of *Turnera elegans* (drawn from a photograph), natural size. The bud *a* opened the following morning. Lower down, two enlarged ovaries can be seen together with the bracteoles. In the case of the two lowest bracts, these have fallen off.

shoot, although very occasionally two may occur together. On an average, each shoot produces a flower about once in 2 days, but the period elapsing between flowering is very irregular. Individual shoots have been observed to produce flowers on four successive days, but periods of several days often occur during which no buds open. The buds which are due to open the following morning can easily be distinguished by their size. The shoot illustrated in Text-fig. 1 was kept with its stem in water and the bud marked *a* opened the following morning. The development of such buds is rapid. The petals begin to emerge from the calyx about 6 p.m., and during the night they elongate rapidly. About 1 hour after dawn the corolla,

which has now become considerably elongated, commences to unroll and the flower opens fully in 1–1½ hours.

The change in the size of the petals during the 24 hours preceding flowering is illustrated in Text-fig. 2. Contact prints on photographic paper were made from petals removed at 8-hour intervals from several buds or flowers at the same stage of development, and the outlines traced from these prints. Owing to the uniformity in development

8.30 a.m.   4.30 p.m.   12.30 a.m.   8.30 a.m.
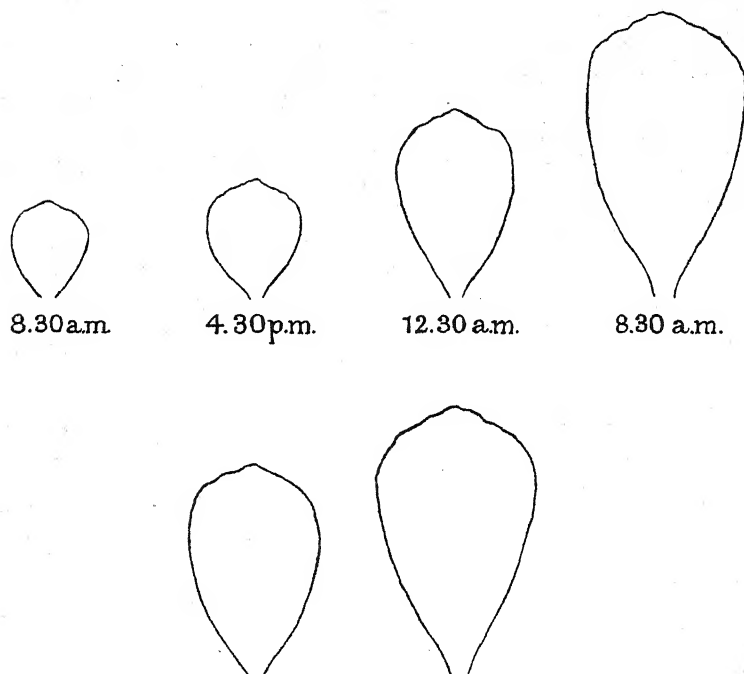
Text-fig. 2. Upper row: actual size of petals at 8-hour intervals up to the time when the flowers opened.
    Lower row: sizes of petals picked at 4.30 p.m. and 12.30 a.m. respectively and left floating on water until 9 a.m. the following morning.

of the flowers, the variation was very slight. For the purpose of the diagram a tracing was made from the print which appeared to represent most nearly the mean size at each stage of development. The outlines in the upper row of Text-fig. 2 represent the actual sizes of petals picked at the times stated. At 4.30 p.m. and 12.30 a.m., petals at a similar stage of development to those illustrated in the upper row were picked and left floating on water until 9 a.m. next morning. Their outlines were then determined by the method

described above and are shown in the lower row. The petals picked at 12.30 a.m. had expanded to practically the same size as those left on the plant, but those picked at 4.30 p.m. were somewhat smaller. Evidently cessation of food supplies had a more marked effect in the case of those picked at 4.30 p.m. than in the case of those picked later.

In Colombo, where these experiments were carried out, the variation in the time of sunrise is from 5.53 a.m. to 6.29 a.m. During the greater part of the year the flowers open about 8 a.m. and close about noon, but during the months of January and February, when the sun rises later and the mornings are somewhat cooler, the flowers do not open fully until about 9 a.m. and close about 1 p.m.

Some experiments, carried out with cut shoots, showed that even if these were kept in complete darkness during the night and following morning, opening and closing of the flowers took place at about the normal time. The periodicity is therefore maintained for at least 1 day in absence of light. Schmucker (4) obtained similar results with the flowers of *Cereus grandiflorus* which normally open in the evening and close about dawn. When the plants were kept in darkness during the day and exposed to light at night, he found that the normal periodicity was retained, but gradually became weaker until, after a few days, it had been replaced by a new one in which the flowers opened in the morning.

It was also found that if the temperature of the air surrounding the flowers of *Turnera elegans* was decreased by a few degrees at the time when opening was about to occur, the expansion of the corolla was checked and full opening was delayed. On the other hand, a rise in temperature hastened, to a slight extent, the opening of the flowers, but tended to promote earlier withering. With regard to the effect of an increase of humidity, it was found that there was no appreciable difference between the times of opening and closing of flowers on shoots which were in a saturated atmosphere under a bell-jar, and of others which were exposed to the air of the laboratory.

The closing of the flower is accompanied by the death of the corolla. In a moist atmosphere the tips of the petals become semi-transparent owing to injection of the intercellular spaces with water from the dead cells. The death of the petals extends in a basal direction. Near the base of the petal is a triangular patch where the cells of the upper epidermis contain a blue anthocyanin pigment. These cells retain their vitality and are capable of being plasmolysed even after the perianth is completely withered and has been detached.

Lepeschkin(3) mentions the conclusion of Oltmanns that the closing and withering of ephemeral flowers are independent phenomena, and confirmed this with regard to the flowers of *Cichorium Intybus*. The same holds good in the case of *Turnera elegans*. Although the distal portions of the petals often show signs of death before the flower has closed, the actual closing is due to nastic movements of the living tissue. This movement can be inhibited by exposing the flowers to chloroform vapour under a bell-jar. Provided that the concentration of the chloroform vapour is not too high, death of the petals takes place at about the usual time, but the petals die in an expanded condition. Lepeschkin(3) mentions that under cool conditions the inflorescences of *Cichorium Intybus* may open for a second time next morning. So far as my experience goes, the flowers of *Turnera elegans* which have once closed never reopen.

After the flower has closed the calyx and corolla remain in a tightly rolled-up condition. The following morning, about 5 a.m., abscission of the perianth is complete and the calyx and corolla are detached, together with the stamens and the style, which breaks off from the top of the ovary.

The change in starch content of petals and sepals

It was noticed that petals and sepals, while in the bud, contain a large quantity of starch which disappears almost entirely after the flower has opened. A careful study was therefore made of the changes in the amount of starch before, during and after flowering. Petals and sepals were detached at various times and immersed in chloral hydrate iodine. They were mounted in the same reagent and studied under the microscope. Sections of young buds, cut from fresh material on a sliding microtome, were also examined.

(*a*) *Petals.* During the day preceding that on which the bud is due to open, starch is present in large quantities both in the mesophyll and in the epidermis. By 10.30 p.m. it has disappeared from the upper and lower epidermis in the distal half of the petal, but is present in the epidermis towards the base. The mesophyll throughout the petal contains starch, but this is present in much greater quantity towards the base. By 5 a.m. starch has disappeared from the mesophyll below the level of the veins in the distal half of the petal. It has also disappeared from the lower epidermis. At 8 a.m., when the flower opens, the whole epidermis is free from starch and only slight traces are present in the mesophyll of the distal half. A fair amount is still present in the mesophyll towards the base of the petal,

particularly just above the region where the blue pigment is present in the epidermal cells. At 11.45 a.m., when the flower is commencing to close, the distal half of the petal is quite free from starch, but near the base starch is present in cells adjoining, but below the level of, the vascular bundles. The cells which contain starch appear in most cases to be packed with grains, but adjoin cells which are entirely free from starch. After the flower has closed, the amount of starch decreases, but even on the next day, after the calyx and corolla have become detached, some cells closely packed with starch grains can be found near the veins in the basal part of the petiole. It would appear that, for some reason, the hydrolysis of starch in certain cells is inhibited, whilst in neighbouring cells the starch disappears completely. In addition to starch grains crystalline aggregates of calcium oxalate are present in the basal part of the mesophyll, both in the bud stage and later.

(b) *Sepals.* The starch changes in the sepals are similar to those in the petals. In the bud stage starch is present throughout the mesophyll. The amount is small in the distal half but is considerable in the basal half of the sepal. Starch is present in the upper epidermis but is almost entirely absent from the lower epidermis with the exception of the guard cells of the stomata. By the time the flower opens the distal half is free from starch with the exception of the guard cells. As in the petal, the amount of starch continues to decrease, but next morning, after the calyx and corolla have been detached, a small amount of starch is still present in the extreme base of the sepal. The guard cells also retain their starch, although in the distal half of the sepal the reaction for starch in them is very slight. Crystalline aggregates, similar to those in the petal, are present in large numbers in the sepal.

The rapid expansion of the petals seems to be connected with the hydrolysis of the stored starch. This hydrolysis would lead to an increase in the osmotic pressure of the cell sap, unless the sugars produced were removed. Although the petals in the bud contain a large amount of starch, the stored-up food material does not seem to be sufficient to allow of full expansion if the petals are cut off from supplies which would otherwise enter them during the night. An inspection of Text-fig. 2 shows that if a petal is removed at 4.30 p.m. it is unable to reach full size when allowed to expand in water.

In both petals and sepals hydrolysis of starch commences in the distal half and proceeds in the basal direction. It is interesting to note that the death of the petal, which also commences in the distal

half, follows soon after the starch has disappeared and proceeds in like manner towards the base.

<div align="center">

THE EFFECT OF NOCTURNAL ILLUMINATION ON
SUBSEQUENT FLOWERING

</div>

During the course of some preliminary experiments on the effect of temperature on the opening of flower buds, an electric lamp was used as a source of heat. It was noticed that buds which were exposed to the light of this lamp throughout the night failed to open next day. The effect of illumination during the night was therefore investigated further.

A number of experiments were carried out, of which the following are typical examples.

Six shoots with buds due to open next day were cut at 4 p.m., and three were placed with their stems in water under each of two bell-jars. The bell-jars were placed on a table under a 100-watt gas-filled lamp, which had a white shade over it, the bulb being 150 cm. distant from the buds. One bell-jar was covered with a black cloth. The light was switched on just before sunset and was left on during the night. Next morning, at 7.45 a.m., the cloth was removed and the light was switched off. In all cases the corollas had protruded to the full extent, but while those which had been in the dark during the night were commencing to unroll, those which had been illuminated were tightly rolled up, and remained in this condition until the flowers withered. The flowers which had been in the dark opened and closed at the normal times.

It appears, therefore, that in the case of buds which are exposed to light during the night, the corollas, although they emerge from the calyx, are unable to expand. It seemed likely that this might be due to some interference with the normal course of starch hydrolysis. In order to investigate this point, a flower of each batch was removed at 10 a.m. and the petals and sepals were treated with chloral hydrate iodine and examined. The petals which had been illuminated during the night contained large amounts of starch throughout the mesophyll, the amount being greatest immediately distal to the blue patch near the base of the petal. The petals which had been in the dark during the night were almost starch-free with the exception of the normal amounts near the extreme base. There was no obvious difference in the starch content of the sepals.

During the following night the shoots were left in the dark, and next morning the withered flowers had fallen off. The petals were

tested for starch. Those which had been illuminated during the first night still contained a considerable amount of starch in the mesophyll towards the base. The others were starch-free with the exception of a few cells near the veins at the extreme base.

In another experiment the effect of illumination during different periods of the night was investigated. Twelve shoots were picked at 6.30 p.m. and three were placed under each of four bell-jars arranged at the same distance from the lamp as in the previous experiment. One jar was covered with a thick black cloth throughout the night, while another was exposed to the light all night. Of the other two, one was shaded from the light until 12.24 a.m., that is, exactly half-way between sunset and sunrise, and then exposed to the light until morning, while the other was exposed to the light until 12.24 a.m. and then shaded until morning.

Next morning, at 7.45 a.m., the shades were removed. All the corollas had protruded to the full extent. Those which had been shaded all night were just commencing to unroll, but the others remained tightly rolled up. By 9.15 a.m. the former had opened normally while the others remained unchanged. By 10 a.m. the flowers lighted during the first half of the night began to expand and opened fully between 11 and 11.15 a.m. The flowers which had been illuminated throughout the night or during the second half of the night remained closed until they withered. Closing of the flowers which had opened first took place about the normal time, 1.15 p.m., but those which had opened late remained open about half an hour longer. Photographs of examples of each of the four groups were taken at 11.30 a.m. and are shown in Pl. I, phot. 4. The illumination during the second half of the night had obviously a greater effect than that during the first half. The reason probably depends on the fact that during the second half of the night the corolla had protruded further from the calyx and was therefore more affected by the light.

Several experiments were carried out in which plants growing in a bed out of doors were exposed to artificial light during the night. An unshaded 100-watt gas-filled lamp was suspended above the bed at a distance of about 30 cm. from the nearest flower buds. The light was switched on at sunset and kept burning throughout the night.

In one experiment the light was kept on for one night only. Next morning the flowers within 80 cm. of the lamp remained closed although the corollas had protruded to the full extent. Beyond this distance there was a graduated amount of opening, the effect diminishing with distance until at about 3.5 m. from the lamp the flowers

were normal. Flowers within this distance on shorter shoots, which were partly shaded from the light, were unaffected or affected to a less extent. Photographs of the whole bed and of typical flowers picked at 11.30 a.m., at distances of 50 cm., 1, 2, 3 and 4 m. from the lamp, are shown in Pl. I, phots. 1 and 2.

The petals of flowers picked at different distances from the light were tested for starch. Those taken from flowers nearest the light contained a considerable amount of starch, especially in the basal half on the distal side of the blue region. The amount of starch diminished with increasing distance from the lamp, until at a distance of about 3 m. the petals were practically free from starch.

On the second day, after the illumination at night, the effect of the light was still apparent. At 7.45 a.m. it was noticed that a large proportion of the emerging corollas were bent sharply towards the place where the light had been. This effect extended to a distance of 2–3 m. from the site of the lamp. Later, about 9 a.m., those within about 1·5 m. of the lamp opened partially, but the petals had a crumpled appearance as if the distal parts had a smaller power of expansion than the basal parts. The appearance was quite different from that of the flowers which had only partly opened on the previous day. In these the petals merely had their edges rolled inwards. In the zone 1·5–2 m. from the position of the lamp, there were many flowers on all sides which remained unopened, although flowers much nearer had partly opened. The corolla in the unopened flowers was swollen at the base but bunched together towards the apex. These flowers were on longer shoots and may have been better illuminated than many which were nearer and which were partly shaded by neighbouring shoots, but this did not seem to be the whole explanation, as even those flowers which were directly under the lamp position opened to some extent. Beyond about 2·5 m. the flowers opened normally. A photograph of typical flowers, picked at 11 a.m. at distances of 50 cm., 1, 2, 3 and 4 m. from the lamp position, is given in Pl. I, phot. 3. Flowers were also picked at 50 cm., 2 and 4 m. from the position of the lamp and tested for starch. Those at 50 cm. had a considerable amount of starch in the distal part of the mesophyll of the petals in longitudinal areas between the veins. The distribution of starch was quite different from that in petals picked on the morning immediately after a night's illumination, when the greatest concentration of starch was in the basal half just above the blue area. The same result was also found at 2 m., but the amount of starch was much less.

On the next day, that is after two nights' darkness, there were no flowers in a sharply defined circular area about 70 cm. in diameter directly underneath the position of the lamp. Outside this area the flowers opened normally. On the following day, after three nights' darkness, flowering was very profuse in the area about 1 m. in diameter directly below the position of the lamp. Apparently buds, whose opening on the previous day had been inhibited, opened at the same time as those due to open normally on that day. Within this area five shoots had two flowers each, whilst outside this area only one such shoot could be found. This was a tall one about 1·2 m. from the position of the lamp. On the fifth and subsequent days flowering was normal.

In view of the after-effects of one night's illumination, it seemed to be of interest to investigate the effect of exposing the plants to light for several nights in succession. With this object, the same lamp, which was in the same position as before, was turned on every night for a week. On the first day the effect was similar to that already described, and illustrated in Pl. I, phots. 1 and 2. On the second the flowers in a circular area about 2 m. in diameter beneath the lamp failed to open, with the exception of a few which were shaded from the direct light and which opened partially. As on the second day of the previous experiment, some flowers 2–2·5 m. from the lamp remained closed with the petals in a crumpled condition. On the third day an area 1·5 m. in diameter under the lamp was flowerless, with the exception of four shoots which were short and therefore partly shaded. On the remaining shoots in this area the corollas did not even emerge from the buds. In a narrow zone outside this area the flowers were half open, and beyond this distance, up to 3–4 m., the flowers which were freely exposed to the light had their petals partly rolled up. On the fourth day the appearance was like that of the first day. A fair number of corollas had protruded in the area about 1·3 m. in diameter under the light, but they did not unroll. Outside this area there was the usual graduated effect. On the next 3 days the effect was similar, but the number of flowers with protruded, but unopened, corollas in the area under the lamp showed a tendency to diminish.

After the nightly illumination had been stopped the effect persisted for a few days. During the first 2 days the flowers near the position of the lamp showed a certain amount of curling inwards of the edges of the petals, but the effect was much less than it was in the previous experiment after one night's illumination. A remarkable

feature was the profusion with which flowers were produced in the area immediately under the lamp. This reached a maximum on the third day. Within a distance of 1 m. of the lamp position there were found on the first day after the illumination had been stopped, twenty-nine double-flowered shoots, on the second twenty and on the third fifty-one two-flowered shoots, twelve three-flowered shoots and one four-flowered shoot. During these 3 days only two other double-flowered shoots could be found in the whole bed beyond this distance. After four nights' darkness flowering became normal.

In view of the small intensity of the light which produces an effect on the flowers, it is of interest to compare this intensity with sunlight and with the light of the moon. Shaw (5) quotes measurements of the illumination of the sun at a latitude of 39° N. and at an altitude of 526 m. For a clear sky in June the illumination was 10,000 foot-candles. The illumination at the latitude of Colombo would be somewhat greater, but no figures are available. With regard to moonlight, the value quoted for zenithal full moon is 0·02 foot-candles. Assuming that the light of the 100-watt lamp was 200 candle-power, then the intensity at 80 cm., which was about the maximum distance at which the expansion of the corolla was completely inhibited in the outdoor experiments, would be about 29 foot-candles, that is, about 0·3 per cent. of full sunlight. The maximum distance at which the effect of the lamp could be detected was about 3·5 m. At this distance the intensity would be about 1·5 foot-candles, that is, about 0·015 per cent. of full sunlight, but seventy-five times as great as full moonlight. It is obvious, therefore, that no effects can be expected after moonlight nights.

The experiments which have been described show clearly that illumination of these plants during the night has a remarkable effect on subsequent flowering. The effect on the flowers seems definitely to be correlated with a partial inhibition of the starch hydrolysis which normally takes place before and during the opening of the flower. The fact that flowers, which have been exposed to light during the night before they are due to open, retain a considerable amount of starch in their petals, even after they have become withered and detached, shows that this inhibition is not merely a temporary after-effect, but persists until the death of the flowers. A further point of interest is that, in addition to the buds which were due to open on the morning after the night's illumination, those due to open on the following day were also affected, provided they were within

2–3 m. of the lamp. In these buds the petals would have been completely enclosed by the calyx during the whole period of artificial illumination and, although these buds were exposed to normal conditions during the following day and night, they did not open properly and the petals possessed an abnormal concentration of starch, particularly in the distal region. On the third day, both in the experiment in which the plants were illuminated for one night only and in that in which the lamp was turned on every night for a week, there were no flowers in the area underneath the lamp where the light had been most intense. The only explanation which appears to be feasible is that the absence of the dark period disturbs some internal rhythm which controls the development of the flower-buds and the formation of diastase.

The fact that the activity of the diastase in the petals is much less when the buds have been exposed to light during the previous night is shown by the following experiment. A lamp was suspended over the middle of the flower-bed in the manner already described and kept on during the night with the usual result. Between 9.15 and 9.45 a.m. 180 flowers, with protruded but rolled-up corollas, were picked from the area under the lamp and the same number were taken from the ends of the bed where the flowers were normal and had opened to the full extent. The corollas were removed in each case and placed in a beaker with a little toluene. After about an hour the sap was extracted by pressure. 2 c.c. of each sample of sap was added to 4 c.c. of 0·5 per cent. starch solution and, at intervals of 5 min., a few drops of each mixture were tested with iodine. At room temperature (29·0–29·6° C.) the sap from the normal flowers had caused complete hydrolysis of the starch to dextrin in 30 min., but the sap from the flowers which had been under the lamp required 120 min. to carry out the same change.

It is apparent, therefore, that the sap from flowers which have been exposed to light during the previous night is very much less active in causing hydrolysis of starch than that obtained from normal flowers. Although Green (2) has shown that extracts containing diastase, which were exposed to sunlight for 2–11 days, became considerably weakened in their action on starch, it is inconceivable that the weak light to which the flower-buds were exposed during the night could have caused destruction of diastase, since full sunlight falling on the flowers and flower-buds during the day does not have this effect. It is much more probable that, as suggested above, the disturbance of the normal alternation of light and darkness partially

inhibits the formation of an active diastase at the time when the buds are due to open.

After these experiments had been completed, the writer became acquainted with the work of Sigmond(6) who found that if flower-buds of *Hedera helix* L. were illuminated during the night, they failed to open next morning. A period of darkness of at least $5\frac{1}{2}$ hours was necessary to enable the buds to open. Flowers which had been prevented, by artificial light, from opening the first morning, opened the following morning, provided normal conditions had been restored. In this respect they differed from the flowers of *Turnera*, which under the same conditions became withered and detached without opening. It must be remembered, however, that in the latter, after one night's illumination, the corolla protrudes at the normal time but does not unfold, whereas the ivy flower remains completely closed. Sigmond suggests that the normal alternation of day and night causes rhythmical changes in growth and turgor and that, if the last dark period is abolished by the use of artificial light, this rhythm is destroyed and flowering is suppressed. Later(7) he carried out experiments on flowers of *Oenothera*, which normally open in the evening, and found that in the case of cut shoots a short continuous darkening before flowering did not affect the opening, but if they were kept for 24 hours in darkness flowering was suppressed. These experiments on *Oenothera* were criticised by Stomps(8) on the ground that the cut shoots suffered from lack of assimilates when kept in the dark. He himself found that intact plants of *Oenothera* continued to produce flowers for 6–8 days in complete darkness.

It is obvious that there are still many problems in connection with this subject. Further work is being carried on by the author, and it is hoped that the results will shortly be available for publication. It may, however, be mentioned here that the inhibitory effect of nocturnal illumination on flowering has been found to obtain in several other plants in which, also, the effect is associated with a varying degree of inhibition in the hydrolysis of starch in the petals.

THE CHANGES IN DRY AND FRESH WEIGHTS OF THE FLOWERS BEFORE, DURING AND AFTER OPENING

(a) *Dry weights*. In order to investigate the flow of food materials into and out of the flower, a study was made of the changes in dry weight over a period of 44 hours, starting 24 hours before the flower opened, and ending when abscission had taken place. For this purpose flowers were taken from plants which had been propagated

vegetatively by cuttings from a single plant. The plants were grown together in one flower-bed just outside the laboratory and, at the time of the experiment, 500–600 flowers were being produced daily.

The method adopted for dealing with each sample was as follows: The flowers were picked and brought at once to the laboratory. Each flower was cut across with a razor immediately above the level of the bracteoles, that is, at the point where abscission would subsequently have taken place. The ovary was removed with a pair of fine forceps, but the styles, which separated easily, were left in the flower. The object in removing the ovary was to limit the investigation to those parts of the flower which are discarded after flowering. Each sample consisted of thirty flowers and was divided into six equal groups.

For the purpose of determining the dry weight the flowers were dried in a ventilated electric oven at 55° C. for 9 hours. In order that they might be exposed evenly to the heated air and prevented from sticking together the following arrangement was adopted. A number of bottle corks were taken and five headless pins were stuck horizontally round the narrow end of each. The corks were put standing on their wider ends and a flower was placed on each pin. The corks were then put into the oven. After drying, each set of five flowers was transferred immediately to a stoppered weighing bottle and weighed.

Preliminary experiments had shown that at 100° C. a constant dry weight could not be obtained, neither with flowers which had opened nor with buds. Owing to the loss in weight which takes place at higher temperatures, Archbold[1] recommends the drying of plant tissues at 50° C. and found that this gave results accurate to within 2 per cent. if the tissues were dried for a sufficient length of time. In the case of the flowers of *Turnera elegans*, it was found that if they were dried for at least 6 hours at 55° C. the weight became practically constant, the loss in weight after a further 18 hours' drying being less than 0·4 per cent. In the present experiment, the conditions for drying were not so favourable as in the preliminary experiment, owing to the larger number of flowers which had to be dried in the oven at the same time, but in two cases where samples were put back in the oven after weighing, for a further 4 hours, the additional loss was less than 1·4 per cent. Owing to the large number of samples which was being dealt with, and to the limited capacity of the drying oven, it was not practicable to allow a longer period for drying than 9 hours, but the error involved is very small com-

pared to the changes in dry weight which occurred during the period of the experiment, and would have affected all the samples to about the same extent.

As each sample was divided into six lots, which were weighed separately, it was possible to obtain an estimate of the standard error of sampling. When this is calculated from all the results it amounts to 1·31 per cent., the highest error in any sample being 2·31 per cent. of its mean value.

The mean weights of a single flower at different times are given in Table I, and the results are plotted in Text-fig. 3.
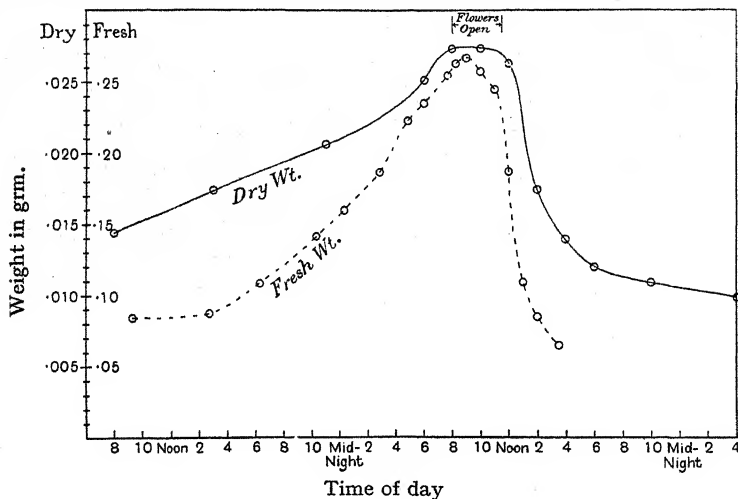
Text-fig. 3. Graphs showing the changes in the mean dry and fresh weights of a single flower before, during and after opening.

(*b*) *Fresh weights.* The change in fresh weight was also studied. As the number of flowers produced in 1 day was insufficient for the simultaneous determination of both dry and fresh weights, the fresh-weight determinations were started 1 day later. The weather was dry and sunny on both days and the flowers opened and closed at about the same time, so that the results are comparable.

For the determination of fresh weights, each sample consisted of thirty flowers which were collected into a closed vessel and brought into the laboratory immediately. They were cut across at the base in the manner already described and the ovaries were removed as rapidly as possible. The flowers were then placed in fives in stoppered bottles and weighed.

From these weights the standard error was calculated for each sample. Owing to irregularity in the rate of loss just after the flowers had closed, the error in the sample picked at 1 p.m. increased to 4 per cent. of the mean, but the standard error calculated from all the samples was only 1·5 per cent. The results of these determinations are given in Table I and plotted in Text-fig. 3.

TABLE I

Mean weight of a single perianth

Dry weight			Fresh weight		
Time	Weight grm.	Standard error	Time	Weight grm.	Standard error
8.00 a.m.	0·01442	0·00011	9.20 a.m.	0·0845	0·0006
3.00 p.m.	0·01746	0·00023	2.45 p.m.	0·0881	0·0006
11.00 ,,	0·02062	0·00027	6.20 ,,	0·1094	0·0010
6.00 a.m.	0·02510	0·00021	10.20 ,,	0·1420	0·0016
8.00 ,,	0·02727	0·00023	12.20 a.m.	0·1601	0·0015
10.00 ,,	0·02729	0·00029	2.50 ,,	0·1869	0·0015
12.00 noon	0·02623	0·00030	4.50 ,,	0·2230	0·0019
2.00 p.m.	0·01741	0·00040	6.00 ,,	0·2346	0·0030
4.00 ,,	0·01396	0·00020	7.40 ,,	0·2541	0·0020
6.00 ,,	0·01199	0·00015	8.15 ,,	0·2625	0·0034
10.00 ,,	0·01091	0·00020	9.00 ,,	0·2662	0·0039
4.00 a.m.	0·00985	0·00018	10.00 ,,	0·2571	0·0023
			11.00 ,,	0·2441	0·0038
			12.00 noon	0·1865	0·0054
			1.00 p.m.	0·1092	0·0044
			2.00 ,,	0·0848	0·0006
			3.30 ,,	0·0649	0·0010

An inspection of the graphs (the dry-weight graph is drawn on a scale ten times greater than that of the fresh weight) reveals several features of interest. Although the flowers are open for a few hours only, the increase in dry weight during the preceding 24 hours is very marked. As starch is stored abundantly in the cells it may be assumed that this increase in dry weight is mainly due to the entry of carbohydrates. The dry weight rose at a steady rate from 8 a.m. until midnight, but the fresh weight did not begin to rise appreciably until the late afternoon. This latter result is to be expected as, during the day, plants exposed to a tropical sun often suffer from shortage of water, as is shown by the slight wilting of the leaves. Between midnight and 8 a.m. both dry and fresh weights rose at a more rapid rate than in the earlier part of the night. Part of the greater rate of increase in the dry weight can be explained by the hydrolysis of starch into sugar of a higher molecular weight, since this hydrolysis takes place actively during this period, but the increase in weight is too great to be explained entirely in this way and indicates an increase in the rate of entry of carbohydrates. The greater rate of

increase of the fresh weight is in accordance with the rapid expansion of the petals which takes place during this period.

One must conclude that the chief function of the sugars formed by the hydrolysis of the stored starch, together with the sugars which continue to enter the flower, is to aid the expansion of the corolla by raising the osmotic pressure of the cell sap.

The maximum dry weight is constant for about half the time during which the flowers are open, but begins to fall before they have closed. The fresh weight, however, commences to fall almost immediately after the maximum has been reached. During the afternoon, both dry and fresh weights fall at a very rapid rate.

The determination of the fresh weights was discontinued at 3.30 p.m., as it was felt that after that time the rate of loss from the dead tissue would depend mainly on atmospheric conditions. The dry-weight determinations were continued until 4 a.m., when abscission was complete and the perianths could be detached by a touch. After about 6 p.m. the rate of loss of dry weight was very small.

The change in dry weight of the ovary after flowering

In the experiments just described the ovaries were removed for the reason stated. It appeared to be of interest to investigate the change in dry weight of the ovary during the period when loss of weight from the perianth is very rapid. For this purpose 150 ovaries were removed from open flowers at 9.30 a.m., divided into three lots of fifty each, and dried on watch glasses for 24 hours at 55° C. Another 150 ovaries were removed at 9.30 p.m. and treated in the same way. After weighing, they were dried again for a further 24 hours, but the additional loss in weight was less than 0·2 per cent.

The results of this experiment are given in Table II.

Table II
Dry weights of fifty ovaries

	Picked at 9.30 a.m. grm.	Picked at 9.30 p.m. grm.
	0·0504	0·0625
	0·0493	0·0632
	0·0500	0·0633
Mean	0·0499	0·0630

The increase in dry weight of a single ovary is therefore 0·000262 grm., which represents only 1·6 per cent. of the loss from the perianth during the same period.

After the flowers have withered the ovaries continue to increase in size, but in most cases they drop off during the following week. The flowers of *Turnera elegans* exhibit the phenomenon of heterostyly, but in the bed from which the flowers used in these experiments were taken only short-styled flowers were produced. In this bed ripe fruits were rare, but in another bed in which both long and short-styled flowers were present they were of frequent occurrence.

UTILISATION OF THE FOOD MATERIALS OF THE PERIANTH AFTER FLOWERING

From the values given in Table I it will be seen that the dry weight of a single flower which was 0·02729 grm. at 10 a.m. had decreased to 0·00985 grm. 18 hours later when abscission had occurred, that is, it had lost 64 per cent. of its dry weight. A small part of this loss might be due to respiration, but the greater portion must represent a loss due to backward flow of food materials into the plant.

In the following experiment an attempt was made to determine the dry weights more accurately and to obtain an estimate of the loss due to respiration. This experiment was carried out in September and, as the flowers appeared to be slightly more variable in size than in the previous April when the results given in Table I were obtained, special precautions were taken to reduce the error of sampling. Well-developed flowers were chosen in every case. In order that these could be distinguished after they had withered, about seventy shoots with suitable flowers were selected at 9.30 a.m. and marked by fastening strips of paper round them. The flowers on the marked shoots were picked the following morning. At 10 a.m. 120 flowers were picked. After removal of the ovaries they were placed on pins stuck into corks in the manner already described. Twelve corks, each supporting five flowers, were then put into the oven at 55° C. and dried (group A), the other twelve were left exposed to the air of the laboratory until the following morning (group B). At 5 a.m. separation of the withered flowers was practically complete and they came off at a touch. Sixty of these were removed from shoots which had been marked on the previous day and put on pins (group C). They were then put into the oven together with the sixty which had been left exposed to the air of the laboratory. The latter appeared slightly moister than the flowers which had been left on the plant. Each group of flowers was dried in the oven for 11 hours and weighed. The weighing bottles, with the stoppers removed, were then placed

in a vacuum desiccator over phosphorus pentoxide at room temperature (27–30° C.). The flowers were dried in this way for 5–6 days, until the additional loss after 24 hours' drying was less than 0·2 per cent. When dried *in vacuo* over phosphorus pentoxide the flowers, which had been previously dried in the oven at 55° C., showed a further loss in weight of 2·0–4·2 per cent. The dry weights of the samples belonging to the different groups are given in Table III.

TABLE III

Dry weights of samples of ten flowers

	A grm.	B grm.	C grm.
	0·2939	0·2906	0·1086
	0·2714	0·2834	0·1078
	0·2785	0·2687	0·1101
	0·2865	0·2818	0·1057
	0·2860	0·2760	0·1079
	0·2912	0·2774	0·1128
Mean	0·2846	0·2796	0·1088
Standard error of mean ...	0·00340	0·00304	0·00099

A, picked at 10 a.m., dried immediately.
B, picked at 10 a.m., exposed to air until 5 a.m., then dried.
C, picked at 5 a.m., dried immediately.

In group B, backward transport of food materials was prevented and the loss in weight would be entirely due to respiration. Owing to hydrolysis of the residual traces of starch which took place after the flowers were picked, the loss due to respiration would be masked to some extent. After 10 a.m. the amount of starch which had not already been hydrolysed would be very small, but if one assumes that 20 per cent. of the dry weight at 10 a.m. is represented by starch which is completely hydrolysed to dextrose by 5 a.m. next morning, then the increase in dry weight for a sample of ten flowers would amount to 0·0063 grm., which is of the same order as the observed loss of 0·0050 grm. Leaving out any correction for hydrolysis of starch, the calculated loss for respiration hardly exceeds the standard error of sampling, and even if this loss were doubled, it would still be negligible compared with the difference in weights between groups B and C, which represents the amount of material passed back into the plant. In Table IV the results are calculated for a single flower and as percentages. It will be seen that 60 per cent. of the weight of dry material in the open flower is transmitted back to the plant, the remainder being used in respiration or lost when the withered perianth is discarded.

It is well known that, in the case of deciduous trees, soluble substances are withdrawn to a large extent from the leaves before these are shed. There can be little doubt that this occurs also in most flowers before the calyx and corolla become withered, but, so far as the writer is aware, quantitative data have not previously been recorded.

TABLE IV

Utilisation of dry material contained in a single flower

	grm.	Standard error grm.	% grm.
Original weight	0·02846	0·00034	100·00
Respired	0·00050	0·00045	1·76
Passed back	0·01708	0·00032	60·02
Lost	0·01088	0·00010	38·22

THE EFFECT OF THE REMOVAL OF FLOWERS ON SUBSEQUENT FLOWERING

The fact that 60 per cent. of the dry material in a flower is restored to the plant before the flower is shed must be of considerable economic importance to the plant. Looked at in another way it means that the amount of material which would be used up by two full-sized flowers (neglecting the ovaries), if this saving did not occur, would under the existing arrangements be sufficient for five. It was therefore of interest to determine what the effect on subsequent flowering would be if the flowers were cut off at the time when their dry weight was at a maximum, thus preventing the utilisation of any of their stored food by subsequent flowers.

For this purpose twenty healthy shoots in the one flower-bed were selected, and numbered labels were attached. These shoots were then observed every morning for 12 days and a record was made of those which flowered. At the end of this period, the shoots were divided into ten pairs in such a way that the members of each pair had produced approximately equal numbers of flowers and were situated in the same part of the flower-bed. The two members of each pair were then differentiated by labels into two groups, A and B. Records were made each day of the flowering of the members of each group and the size of each flower was estimated by measuring with a rule the diameter of the open flower, taking the mean of two measurements at right angles. Observations and measurements were made about 9 a.m. each day, that is, about 1 hour after the flowers had opened, and were continued for a further period of 12 days.

In group A the flowers were allowed to wither on the shoots, but the ovaries of those which had opened on the previous day were cut off about 9 a.m.

In group B the open flowers, including the ovaries, were cut off at 9 a.m.

The essential difference between the treatment of the two groups is that in group A the food materials from the withering flowers were retained in the shoots, while in group B they were removed at the time when the maximum was present in the flower. As the ovaries were cut off in group B when the flowers were removed, they were also cut off in group A after the flowers had withered, to prevent diversion of food materials into them.

The results of this experiment are given in Table V.

TABLE V

Preliminary period				Experimental period			
	No. of flowers produced				No. of flowers produced		Mean diameter of corolla in cm.	
Days	Group A	Group B		Days	Group A	Group B	Group A	Group B
1	5	7		13	7	7	5·1	4·8
2	6	4		14	4	4	?	?
3	5	5		15	7	8	4·8	4·5
4	4	3		16	3	3	5·0	4·3
5	5	8		17	7	8	?	?
6	5	3		18	4	2	5·2	4·9
7	5	6		19	5	6	4·6	4·7
8	5	4		20	3	3	4·9	4·9
9	4	4		21	2	5	5·1	4·9
10	7	6		22	7	5	4·9	5·1
11	5	5		23	2	2	4·9	4·7
12	3	3		24	5	6	5·2	4·7
Total	59	58			56	59		
Mean							4·97	4·75

It will be seen that the removal of the flowers when fully open does not affect the rate at which subsequent ones are produced, as the total number of flowers which open over a period of days remains approximately constant. With regard to size, there appears to be a tendency for the flowers in group A to be slightly larger than those in group B owing to the conservation of food material. On 7 days out of 10 (on the two remaining days the flowers were damaged by heavy rain and measurements were impracticable) the diameter of the flowers at full opening in group A was slightly greater than in group B, but the difference is small and cannot be regarded as significant. In the next experiment, however, it is very strikingly shown

that starvation of the shoots does affect the size of the individual flowers, while the rate at which they are produced remains constant until food supplies are exhausted.

THE EFFECT OF STARVATION ON SUBSEQUENT FLOWERING

Ten healthy shoots with buds due to open the following morning were picked and brought into the laboratory. Each had been flowering for several months, so that the upper 30 cm. or so had no foliage leaves but only persistent bracts. The shoots were cut under water to a length of about 20 cm. and placed with the cut ends in vessels of water in front of a window. Although cut shoots of *Turnera elegans* retain their vitality for a long time when placed in water, it was obvious that the bracts when exposed to the diminished light of the laboratory would not be able to form sufficient carbohydrates to supply the needs of the developing flower-buds. Records were made of the number of flowers produced each day, and the diameter of each open flower was measured. These results are given in Table VI.

TABLE VI

Days	1	2	3	4	5	6	7	8	9	10	11	Total
No. of flowers produced	10	3	7	1	7	6	2	8	0	3	7	54
Mean diameter in cm.	5·7	5·0	5·3	4·7	4·2	4·3	3·5	3·6	—	2·8	2·6	

No flowers were produced on any of the shoots after the eleventh day. After this period the shoots remained alive for more than a week, but the younger buds fell off unopened. During the first 11 days each shoot produced a total of either five or six flowers. The rate of flower production showed no tendency to decrease and the total number of flowers produced, fifty-four for ten shoots in 11 days, agrees very closely with 56–59 for the same number of shoots in 12 days on plants growing outside (cf. Table V). It is obvious, therefore, that starvation does not affect the rate at which flowers are produced. This rate would appear to depend on some internal rhythm which is maintained until lack of food material brings it to a sudden end. Starvation does, however, have a marked effect on the size of the flowers. The diameter of the flowers decreased fairly steadily, until at the end of the period they were about half the normal size. From the eighth day onwards some of the flowers were unable to open completely of their own accord, apparently owing to lack of turgidity in the petals. These were opened by hand in order that approximate measurements of their size could be made.

It appears, therefore, that although the later flowers opened at the ordinary times, lack of carbohydrates prevented the full expansion of the petals. This confirms the previous results which showed that the normal expansion of the corolla is associated with the hydrolysis of starch to sugar. This change raises the osmotic pressure and allows the petals to become turgid. The necessity for the increased osmotic pressure would obviously be greater in the case of whole plants growing out of doors than in the case of cut shoots, as in the former the entry of water into the petals would be opposed by the tension which is set up in the water columns during the day. Schmucker(4) suggested that such carbohydrate changes might be responsible for the opening of the flowers of *Cereus grandiflorus* and that the utilisation of the sugars during anthesis, as indicated by a slight rise in temperature, could, at least in part, explain the closing and drooping movements which commence some hours later. This latter suggestion is improbable. As was mentioned previously, the death of the flower of *Turnera elegans* is due to definite lethal changes, and the dry weight does not begin to fall appreciably until death has set in. The loss due to respiration of detached flowers was found to be very small, and the bulk of the soluble carbohydrates is drawn back into the plant while the flowers are withering.

Summary

1. *Turnera ulmifolia* L. var. *elegans* Urb. produces, throughout the year, flowers which open about 2 hours after sunrise and close 3–4 hours later. The closing of the flower is accompanied by the death of the corolla, and abscission of the perianth occurs early the following morning. If the flowers are kept in the dark during the morning they open and close at about the ordinary times. Variations in temperature have an influence on the rapidity of opening, but the times at which opening and closing occur are not affected by an increase in the humidity of the air.

2. The petals and sepals in the buds contain large quantities of starch which is hydrolysed before and during the opening of the flowers. The rapid expansion of the petals appears to be connected with the hydrolysis of the stored starch.

3. Illumination of the plants during the night has a marked effect on the opening of the flowers next morning. The corollas of flowers which were within 80 cm. of a 100-watt lamp remained rolled up, and up to a distance of about 3·5 m. the amount of opening was dependent on the distance from the light. The action of the light

appears to be correlated with an inhibition of the normal hydrolysis of starch in the corolla. The after-effects of one night's illumination are not limited to the following morning but persist for a few days.

4. Changes in the fresh and dry weights of the flowers were studied before, during and after flowering. Both fresh and dry weights rise fairly steadily until the flowers open, but after these have closed they fall very rapidly.

5. About 60 per cent. of the dry material of the perianth passes back into the plant before the flower is shed. The material thus saved is therefore available for the production of subsequent flowers.

6. The effect of lack of food material on the production of flowers was studied. Although the size of the flowers is decreased by starvation, the rate of production appears to be determined by some internal rhythm, and remains constant until lack of food brings it to a sudden end.

I am indebted to my wife for valuable assistance in the determinations of the changes in dry and fresh weights and in other experiments in which large numbers of flowers had to be picked and dealt with in a short space of time.

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EXPLANATION OF PLATE I

Phot. 1. Appearance of flower-bed after one night's illumination by 100-watt lamp.
Phot. 2. Flowers picked from above bed at distances of 50 cm., 1, 2, 3 and 4 m. from the lamp.
Phot. 3. Flowers picked at similar distances on the following morning, after one night's darkness.
Phot. 4. Left to right: 1, exposed, indoors, all night to 100-watt lamp; 2, exposed first half of night; 3, exposed second half of night; 4, shaded all night.
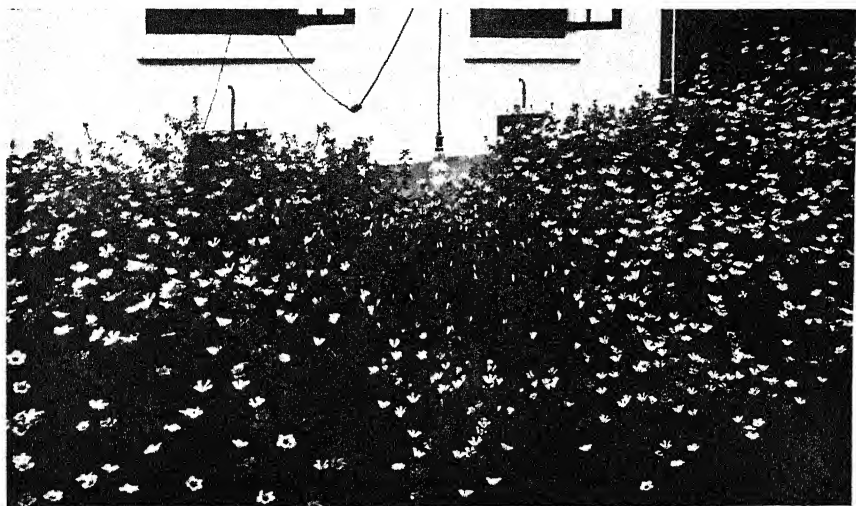
Phot. 1
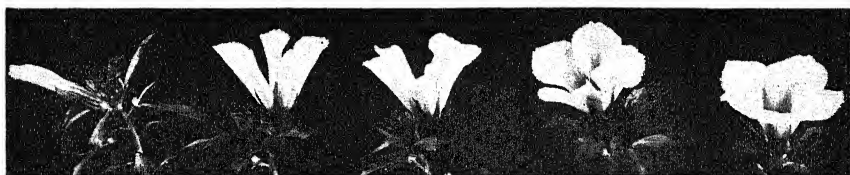
Phot. 2

Phot. 3
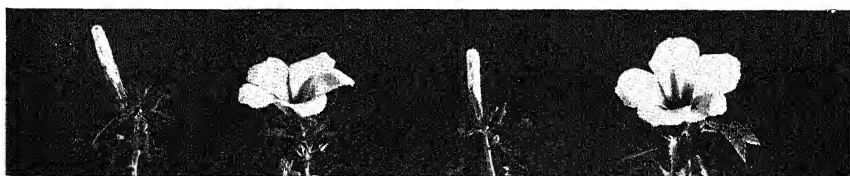
Phot. 4

BALL—EPHEMERAL FLOWERS OF *TURNERA ELEGANS*

THE RÔLE OF THE ORGANIC ACIDS IN PLANT METABOLISM

PART I

By T. A. BENNET-CLARK

From the University of Manchester

(With 3 figures in the text)

CONTENTS

MODE OF OCCURRENCE OF THE PLANT ACIDS

ALTHOUGH the first acids known to man were probably those produced in fruits and vinegar fermentations, even at the present day little definite information is available as to the manner in which these acids are formed by plants, or as to their functions in plant metabolism.

It is known now that it is only in exceptional cases that the reaction of the cell sap of plants is not on the acid side of the neutral point, and very commonly the acidity is quite considerable. The commonest of the acids which are responsible for this acid reaction are oxalic, malic and citric, and it is with the occurrence and metabolism of these acids that this account deals. These acids are normally present in plants in the form of their salts. The actual concentration of free acid is usually rather low. This circumstance has given rise to a number of erroneous and ambiguous statements which are commonly found in the literature. For example, Hempel[24] in describing the acid metabolism of the Crassulaceae makes the statement: "the acidity...is due to a mixture of acid salt and normal salt." Again, Small writes[39]: "within a certain pH range a malate system will be all acid salt plus normal salt."

Statements such as these give the erroneous impression that the free acid does not occur in such solutions as plant saps, which could be imitated by dissolving the acid salt and the normal salt in water. However, such solutions are bound to contain the free acid in equilibrium with other ionic species. These solutions will contain the cations of the base (B·) formed on the dissociation of the salts, divalent anions (A″) formed from the neutral salt, and univalent anions (HA′) formed from the acid salt: but these univalent ions dissociate further, and in addition react also with the hydrogen ions formed by this dissociation, so that the following reactions take place:
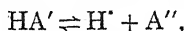
$$HA' \rightleftharpoons H^{\cdot} + A'',$$
$$HA' + H^{\cdot} \rightleftharpoons H_2A.$$

Any solution, therefore, which "contains the acid salt" of a dibasic acid consists of an equilibrium mixture of the following ionic and molecular species: B·, H·, A″, HA′, and H_2A. The fraction of the total acid which is present in the form of free undissociated acid can be shown to be quite simply related to the hydrogen-ion concentration. The undissociated fraction or dissociation residue (r) is equal to

$$\frac{[H_2A]}{[H_2A] + [HA'] + [A'']} \qquad \ldots\ldots(1),$$

the equilibrium concentrations of the various species are given by the dissociation constants of the acid thus:

$$\frac{[HA'] \cdot [H^{\cdot}]}{[H_2A]} = k_1 \qquad \ldots\ldots(2),$$

$$\frac{[A''] \cdot [H^{\cdot}]}{[HA']} = k_2 \qquad \ldots\ldots(3),$$

whence $[HA'] = \dfrac{k_1 \cdot [H_2A]}{[H^{\cdot}]}$ and $[A''] = \dfrac{k_2 \cdot k_1 \cdot [H_2A]}{[H^{\cdot}]^2}$,

and substituting these values in (1), one obtains

$$r = \frac{[H_2A]}{[H_2A] + \dfrac{k_1 \cdot [H_2A]}{[H^{\cdot}]} + \dfrac{k_2 \cdot k_1 \cdot [H_2A]}{[H^{\cdot}]^2}},$$

whence, dividing by $[H_2A]$

$$r = \frac{1}{1 + \dfrac{k_1}{[H^{\cdot}]} + \dfrac{k_1 \cdot k_2}{[H^{\cdot}]^2}}.$$

The relationships between the hydrogen-ion concentration and the fraction of the total acid present in the forms of univalent and

bivalent ions are obtained similarly. These are illustrated graphically for the special case of malic acid in Fig. 1. The value of k_1 has been taken as $3 \cdot 95 \times 10^{-4}$ and k_2 as $8 \cdot 3 \times 10^{-6}$. The univalent fraction attains its maximum value at the pH which is the mean of pk_1 and pk_2. In acid solutions the undissociated fraction predominates, and similarly the bivalent fraction predominates in alkaline solutions.

Plant saps in general are more acid than pH 5·5, and in the special cases of the succulent plants, the Polygonales, and certain
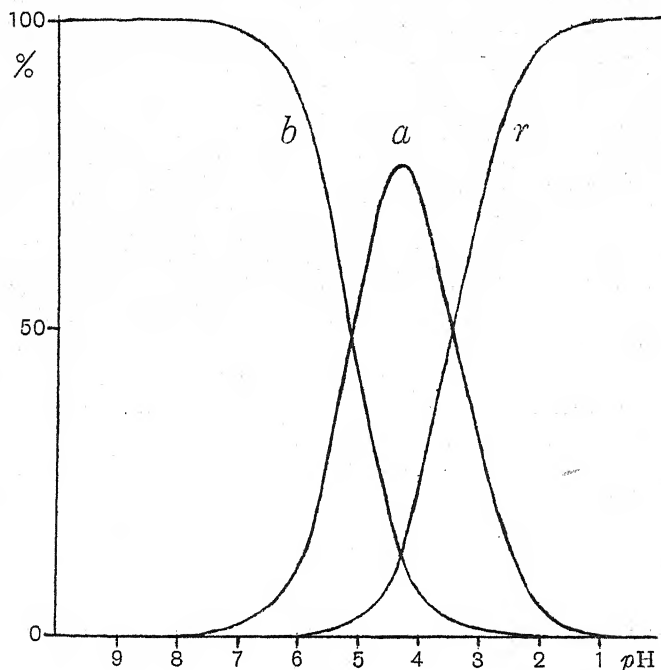
Fig. 1. Percentage of total malate present as M'' (*b*), HM' (*a*), and H$_2$M (*r*) in solutions of various pH.

other groups of plants of which the acid metabolism has been much investigated, the pH values of the saps lie more commonly between about 4 and 5. A small fraction of the total malate present will therefore be present in the form of the free undissociated acid in the sap. The relationships indicated in Fig. 1 may be compared, for example, with Small's statement ((39) p. 294), that at pH 5 no free acid is present in a malate system, but that at a pH of 3·95 "a certain small proportion of free malic acid is present." Actually some 3 per cent. of the total malate is present as free undissociated

acid at a pH of 5 and about 20 per cent. of it is present as free acid at a pH of 3·95.

In acid solutions of the salts of dibasic acids, such as plant saps, we have to discriminate between four quantities which are by no means clearly distinguished in the literature. *Firstly*, the hydrogen-ion concentration, which is very commonly termed the "actual acidity" by German authors. *Secondly*, the concentration of free undissociated acid; the term "free acid" which is frequently found in the literature does *not* refer to the concentration of free undissociated acid, which cannot be directly determined, but which can be approximately deduced from the pH of the solution by the method indicated above. *Thirdly*, the titratable acidity; this is quite commonly referred to in the literature as the "free acid," but this expression is quite erroneous, since the fraction of the total acid which is available for the neutralisation of an added base is equal to the sum of the fraction present in the undissociated state plus half the fraction present as univalent ions. This quantity is available for conversion into various products of metabolism. This is made clear when one considers that if the free undissociated acid is used up, equilibrium will be re-established by the change

$$2HM' \rightleftharpoons H_2M + M'',$$

proceeding from left to right, and conversely if more acid is formed equilibrium will be re-established by the reverse change occurring. *Fourthly*, the total acidity is the total quantity of anions and molecules of the acid and is therefore best expressed as the number of mols or equivalents of the acid. This quantity is obtained by neutralising and precipitating the acid as an insoluble salt. It is frequently termed, by German writers, the quantitative acidity. Finally, the difference between the total acidity and the titratable acidity will be termed the non-titratable acidity; the expression "bound acid" which is sometimes used for this quantity is not strictly accurate.

The confusion between free acid and "available" acid resembles the confusion which used to exist between the quantity of available hydrogen (given by the normality of the acid) and the actual hydrogen-ion concentration. The free-acid concentration, like the hydrogen-ion concentration, is a determinant of the rate of utilisation of the acid rather than of the quantity which is available.

It is clear that ordinarily the quantity of acid formed or used up during metabolism is obtained by determining the increase or decrease in the titratable acidity, and this must be equal to the change in quantity of total acid under most circumstances. In fact the

change of total acidity will exactly equal the change in titratable acidity unless the quantity of base changes at the same time. The only base, of which the concentration has been observed to change rapidly in the tissues, is ammonium, and it is pointed out by Ruhland and his co-workers that many plants which are rich in organic acids are also rich in ammonium salts, and moreover rapid fluctuations in ammonium content apparently take place. Unfortunately, preliminary accounts only have been published, and these deal only with *Begonia* and *Rheum*, so that at present one has no certain knowledge of the distribution of ammonium-rich plants throughout the plant kingdom.

Evidently, if ammonia is formed simultaneously with the formation of acid, there will be a gain in the total acid content, but the titratable acidity will not increase to the same extent and, in fact, may decrease in quantity if more equivalents of ammonia than of acid are formed. It is, however, also clear that the change in total acidity should equal the change in titratable acidity plus the change in ammonium content (rise in concentration being expressed in each case as +, and fall as −). The point is emphasised here because changes in titratable acidity and ammonium content can readily be determined with considerable accuracy, whereas the estimation of the total acidity presents considerable difficulties and is liable to large errors. It is consequently desirable that any observed changes of total acidity should be experimentally verified by determining at the same time the change in titratable acidity and the change in ammonium content, the algebraic sum of which should equal the change in total acid content.

This point is of some importance, since most of the data regarding the acid metabolism of plants have been obtained from the malic acid-containing succulent plants. Now these plants are characteristically very poor in ammonium salts; the ratio of number of equivalents of ammonia/number of equivalents of titratable acid is normally less than $1/100$, and the fluctuation in ammonium content is slight, so the mass of data available as to the changes of titratable acidity in these plants can be accepted as a true account of the changes in total acid content also. This is, however, not the conclusion arrived at by Bendrat(7), working in Ruhland's laboratory. She considered that the titratable acidity yielded no information of value regarding the acid metabolism in succulents since the sign of the change of total acidity was, according to her results, opposite to that of the change in titratable acidity in many cases, and in almost all cases the amount

of change in titratable acid differed from that of the total acid. For example, in a typical experiment ((7) p. 564, no. 74), in which *Sempervivum glaucum* was used, the following changes were recorded:

Total acidity	...	...	...	...	+ 18 %
Titratable acidity ...	...	...	...	− 11 %
Hydrogen-ion concentration		...	...	+ 49 %

The increase in total acidity coupled with decrease in titratable acidity might be explained by the formation of large amounts of ammonia, though it is not known to be formed in such quantities in succulents; but in this case it is quite clear that the explanation of the result is experimental error because an increase in total acid and decrease in titratable acid content must of necessity bring about a decrease and not an increase in hydrogen-ion concentration. The fact that the sign of the change of hydrogen-ion concentration is the opposite of what would be expected from the acidity changes in at least a dozen of the experiments evidently indicates that the experimental technique used for obtaining the total acid or titratable acid content introduces considerable error. Unfortunately this technique is not described, but the various methods for estimating the total acidity which are reviewed later are all liable to rather large errors as compared with those involved in determining the titratable acidity, and one must therefore conclude that the latter measurements form as reliable a guide as is obtainable to the quantitative changes in acidity which occur.

The only base, which can cause rapid disappearance of the titratable acid by neutralising it, is ammonia, but a slow neutralisation of the acid takes place in many plants by bases absorbed from the soil. These bases are in part made available by the conversion of the ions NO_3', PO_4''' and SO_4'' into parts of the protein molecule, but since the number of equivalents of base associated with organic acids often exceeds the number of equivalents of nitrate sulphate and phosphate converted, it is evident that the absorption of cations must be accompanied either by a passage of hydrogen ions from the plant to the soil or by the entry of anions such as hydroxyl or the aluminate ion which would lose their negative charges by interaction with other ions inside the plant.

This slow neutralisation as the plant increases in age is typical of the "malate" plants (such as the Crassulaceae and Cactaceae). In these plants the old parts contain less titratable and more nontitratable acid than the young parts (Astruč (3)).

It follows from the discussion above that the equilibrium mixture of the acid and its ions acts both as a regulator, or buffer, of the hydrogen-ion concentration, and of the concentration of free un-dissociated acid. The importance of this latter "acid-buffering" in metabolism has not been recognised. It is, however, clear that the formation or utilisation of the organic acid from such mixtures as occur in sap will produce very little change in the concentration of the free undissociated acid in the sap. A large change in the titratable acidity will occur, but owing to the large reserve of non-titratable acid (salts of the acid) the change in total acidity will be slight, and the change in pH and consequently of the free acid concentration will also be slight.

Changes in the titratable acidity of a tissue need not therefore be expected to bring about marked changes in the various metabolic processes which are most intimately linked with the organic acids.

Finally, the argument may be recalled here that, because the organic acids are present in plants in high concentration, it follows that they play no active part in metabolism, since only an inactive storage or waste product can survive in high concentration in the tissues. Apart from all the other evidence which will be considered later, the answer to that argument is that the free organic acids are present in quite low concentrations: the concentration of free malic acid, for example, in plant tissues probably rarely exceeds 0·05 per cent. of the fresh weight and is usually much lower, though the total malate content may be as high as 2 per cent. of the fresh weight.

THE DISTRIBUTION OF THE COMMONER PLANT ACIDS

Oxalic acid. Oxalates and oxalic acid are probably present in almost all plants, though in most cases the quantity present is small. All the most careful separations and analyses reveal the presence of traces of oxalate, but plants which are rich in oxalate are relatively few and belong in the main to the following groups.

Polygonales. The genera *Rumex* and *Oxyria* have been recognised as highly acid oxalate plants for a long time. *Rheum* has also been assumed to be a typical oxalate plant (Steinmann[42]), but the recent work of Ruhland and Wetzel[35] has shown that the rhizomes and leaf stalks contain only traces of oxalate but are rich in malate. The leaf laminae contain little oxalate early in the season, but as they age the malate disappears and oxalate appears. The withering leaves in autumn are rich in oxalate.

Centrospermae. This order, which is close to the Polygonales, contains many well-known oxalate plants, which are almost all characterised by their small content of titratable acid and the consequent nearly neutral reaction. Such plants are the succulent halophytes, *Salicornia, Salsola, Atriplex,* etc. The desert succulents belonging to the Portulacaceae and Aizoaceae are similar. Plants belonging to the latter order also contain malic acid; in *Mesembryanthemum cordifolium*(7), for example, the ratio of malic to oxalic acid may be as large as 0·7:1·0, but in older leaves the ratio falls to about 0·25:1·0. Such data are not available for other members of the Centrospermae, but the expectation seems justifiable that the acid metabolism of the Polygonales and Centrospermae resemble that of *Rheum* and *Mesembryanthemum,* the two best investigated genera. The recent work of Thoday and Evans(44) has shown that the malate and oxalate in *Mesembryanthemum* do not occur mixed in the cells but that some cells are rich in oxalate, others contain malate but no oxalate. The significance of this differentiation has not yet been explored.

Geraniales. Geranium, Pelargonium, Tropaeolum, and *Oxalis* are typical acid plants rich in oxalic acid. *Geranium* is rich in malic acid also.

Parietales. Begonia is a genus of highly acid plants containing almost exclusively oxalic acid, traces (0·5 per cent.) of malic acid are also present (Ruhland and Wetzel(34)). The very low pH of the cell sap of the leaves is striking, about 1·5–2·0.

Other plants. The most noteworthy other oxalate-producing plants are the mould fungi, indeed almost all fungi which have been investigated produce oxalic acid, in some cases in very large quantities, so that the media in which they grow may have as low a pH value as 1·5.

The malic acid group. Probably most plants contain malic acid, but the methods which are available for its certain recognition are so laborious that no very extensive survey has been effected, and this applies still more forcibly to the closely related acids, tartaric, succinic, fumaric, and dioxy-maleic. Since malates are so universally distributed the list given below merely records the natural orders and plants in which remarkably large accumulations of malate are found.

Liliiflorae.	Many species, *Agave, Haworthia,* etc.
Farinosae.	Bromeliaceae.
Microspermae.	Most *orchids.*
Polygonales.	*Rheum,* contains oxalic acid also.
Centrospermae.	*Mesembryanthemum,* contains oxalic acid also.
Ranales.	*Ranunculus, Berberis.*
Rhoedales.	*Fumaria, Glaucium,* also contain fumaric acid.

Rosales.	Crassulaceae, Saxifragaceae, Rosaceae, etc.
Geraniales.	Euphorbiaceae, *Geranium* (+ oxalic acid).
Sapindales.	*Fraxinus*, Staphyleaceae.
Rhamnales.	*Rhamnus*, Vitaceae, also contain tartaric acid.
Opuntiales.	All.
Contortae.	Asclepiadaceae.
Campanulatae.	Many Compositae. *Lactuca* also contains succinic acid.

It is noteworthy that plants which are very rich in malate are found distributed amongst many natural orders. The malic acid is known to be associated with traces of the related acids and oxalic acid where detailed analyses have been made, and this is probably always the case.

Large quantities of fumaric acid have been recorded in the case of *Fumaria, Corydalis,* and *Glaucium.* In the latter the ratio of fumaric to malic acid concentration is approximately 2:1. Oxalic and citric acids are also present in large quantity, and traces of dioxy-maleic acid are present (Schmallfuss[38]).

Tartaric is the characteristic acid found in the leaves and fruits of the Vitaceae, but small amounts of malic and succinic acids are also present.

Succinic acid is present in *Lactuca* (Ullrich[45]) in rather large quantity; an approximately equal quantity of malic acid is also present. Both it and fumaric acid are formed in quantity by many mould fungi, particularly the Mucorineae and *Aspergillus fumaricus.*

Further information as to the occurrence of these acids will be found in the series of papers by Franzen and his co-workers[15-23].

Both the laevo- and dextro-rotatory isomers of malic and tartaric acids are found in plants which contain these acids. Laevo-malic and dextro-tartaric acids are the forms which are present in greatest concentration in the tissues.

The citric acid group. Citric acid is chemically related in constitution to malic acid, and at the present time it is thought probable that it is formed from malic acid in plants. It is at all events nearly always found in tissues containing malic acid. It is produced in large quantity accompanied by only traces of other acids (malic, oxalic, etc.) in the *Citrus* fruits (Rutaceae), some members of the Solanaceae, many *Aspergilli*, and most *Penicillia.*

Many fruits contain large quantities of citric acid associated with malic acid[19, 20], amongst these may be mentioned species of *Prunus, Pyrus, Rubus, Ribes, Vaccinium.* Its distribution among certain species of *Prunus* is of interest: *P. spinosa* and *P. domestica* contain malic acid with traces of citric acid; *P. cerasus* contains approxi-

mately equal quantities of citric and malic acids; *P. padus* contains almost exclusively citric acid with traces of malic acid. Whether the relative quantities of the two acids change during the ripening of the fruits has not yet been investigated.

The closely related tricarballylic and aconitic acids have been obtained in the great variety of products isolated from sugar-beet molasses, and the latter is also obtained from *Aconitum*.

Other related acids. For the sake of completeness, it should be pointed out that lactic acid has been obtained from *Rubus*, *Agave*, *Solanum*, and *Glaucium* in considerable quantity. Traces of it are found in *Echeveria* and other succulents. Glycollic acid has been detected in many fruits and other tissues.

Gluconic acid is produced in large quantity by many mould fungi.

METHODS FOR THE DETECTION AND ESTIMATION
OF THE PLANT ACIDS

General methods. The method used by Astruč[3] and Hempel was indirect and of quite limited applicability, although in the special cases which they investigated its use is probably quite justifiable. They estimated the titratable acidity by titration with soda, and estimated the non-titratable acidity by converting the tissue to ash and estimating the number of equivalents of base in the ash. Astruč did this by titrating the ash with standard acid; Hempel[24] estimated the quantity of calcium, magnesium, aluminium + iron, and sodium + potassium separately by standard gravimetric methods. The validity of the method depends on the assumption that all (or almost all) the inorganic cations in the tissue are associated with the organic acid anions: when ashed the salts of the organic acids are converted into the oxides or carbonates of the metals, which can be estimated by titration or gravimetric methods. It is obvious that the ammonium salts if present would be completely lost by this treatment. The presence of phosphate (cf. p. 48) introduces considerable error in some cases.

The method gives a valuable indication, although not a strictly accurate measure, of the total acidity.

Details of the more critical precipitation methods cannot be given here. The most commonly used methods have been the precipitation of the calcium or lead salts of the acids. The calcium salts of all the acids except oxalic acid are soluble in relatively acid solution (pH 3); calcium oxalate is very sparingly soluble but its solubility increases

as the acidity increases. Oxalic acid and oxalates can therefore be readily estimated in the presence of other acids (cf. Bau(5)).

The calcium salts of the common hydroxy and polybasic acids are soluble in water but are very sparingly soluble in 80 per cent. alcohol, and are consequently precipitated by addition of alcohol to the neutral aqueous solution. Calcium acetate, glycollate, glyoxylate, and lactate are soluble in 80 per cent. alcohol. 80 per cent. alcohol precipitates other compounds, such as polysaccharides and some proteins, as well as the calcium salts of the polybasic acids, so that the weight or carbon content of the crude precipitate cannot be ordinarily used as a measure of the acidity. The weight of calcium oxide or sulphate obtained after ignition can be used as a measure of the number of equivalents of acid. Difficulties are, however, frequently encountered, as the presence of various substances in the tissue extracts inhibits the precipitation of the calcium salts, especially that of malic acid; lactic acid, for example, is one of these inhibiting substances. The special methods required for the estimation of malic acid in muscle where the precipitation of calcium and of silver malate is inhibited in this way are described in some detail by Needham(31).

Many plants rich in malates are rich in substances which inhibit the precipitation of calcium malate; the Cactaceae, Liliaceae, Orchidaceae, for example, are rich in mucilaginous polysaccharides (pentosans, etc.) which prevent complete precipitation of malate, and it is invariably necessary to conduct control estimations of the malate in samples of sap to which known amounts of malic acid have been added, in order to find out whether the added acid can be recovered. Unfortunately plant physiologists have neglected to conduct such controls (cf. Needham(31)).

The accuracy of the published records of the total acid contents of plant tissues is therefore suspect.

No such suspicion attaches to the recorded values of the quantities of standard alkali required to neutralise a given quantity of plant material, but in some cases there is difficulty in interpreting these results. When the titration is carried out with phenolphthalein as indicator the alkali is neutralised by the titratable plant acids (malic, citric, oxalic, etc.) and also by weaker acids and certain other compounds. The more important of these compounds are carbon dioxide and phosphoric acid, which are in general present in small quantity, protein-like compounds the nature of which is unknown (see Hempel(24)), and aluminium malate. Since considerable quantities

of aluminium malate are present in some succulent plants, the behaviour of this salt is important. Its solutions are acid owing to the weakness of the base alumina. On titration with soda, alumina is precipitated, and the fraction of the aluminium salt hydrolysed and converted into alumina in this way depends upon the pH to which the solution is titrated. In the case of succulent plant saps approximately 40 per cent. of the aluminium malate is hydrolysed at pH 7 and approximately 90 per cent. is hydrolysed at pH 9·3 (end-point with phenolphthalein).

The significance of this with regard to titrations of succulent plant saps will be made clear by an example. Consider the first experiment recorded in Table I. Since one can assume without much error that all the base is present as malate, it follows that the non-titratable malate content is 22·58 mg. equiv. (in the sense defined on p. 40). On titration of the sap to pH 7, the 4·99 mg. equiv. of aluminium malate yield approximately 2·0 mg. equiv. (40 per cent. of 4·99) of malic acid by hydrolysis; the observed titratable acidity of 5·02 is therefore made up of 2 mg. equiv. derived from hydrolysis of aluminium salt and 3 mg. equiv. which is the titratable acidity in the sense defined earlier (p. 40). Similarly on titration to pH 9·3, 90 per cent. of the aluminium salt is hydrolysed giving 4·5 mg. equiv. of acid, which with the 3·0 mg. equiv. of titratable acid (as defined) accounts for the 7·5 mg. equiv. of soda used when the titration is carried to the end-point of phenolphthalein. In this case therefore the total malate content is about 25·6 mg. equiv.

TABLE I

No. of mg. equiv. per 100 grm. sap (Hempel(24))

Plant	NaOH to bring to pH 7	NaOH to bring to pH 9·3	Al + Fe	Ca	Mg	Na + K
Rochea	5·02	7·49	4·99	9·13	1·73	6·73
falcata	4·21	6·61	6·63	7·36	1·10	6·46
Cotyledon	0·59	2·27	3·03	12·00	0·63	1·71
linguaefolia	1·02	2·41	2·29	11·60	0·76	1·53

Thoday and Evans(43) also point out that in some cases the presence of phosphates influences the results of titrations. Most members of the Crassulaceae are poor in phosphate, but *Kleinia*, for example, especially in the old stems, is rich in phosphate. As the phosphate is neutralised to the tribasic salt on titration to pH 9·3, the titration gives the sum of the titratable phosphate and malate; unless the tissue contains very small quantities of phosphate com-

pared to the malate content this clearly affects the interpretation of the results of the titration. The phosphate is all in the form of bivalent ions (HPO_4'') at pH 4·5, and most of it is still in this form at pH 6. Therefore if the sap is titrated to pH 6 the phosphate does not react with the alkali appreciably, whereas almost all the malic acid does, as Thoday and Evans' titration curves show.

Titrations to this end-point give a rather satisfactory measure of the titratable malic acid (also citric and oxalic acid), which does not need to be corrected for the phosphate and aluminium content of the cell sap. The writer has found that chlor-phenol red is a good indicator for this end-point: titrations must of course be done in a comparator.

In conclusion one may point out that in the study of acid metabolism the chief requirement is a knowledge of the amounts of acid formed or used, and therefore the change in titratable acidity or total acidity must be known. Since the total acid content greatly exceeds the titratable acidity the percentage change in the latter is greater in the course of metabolism; sampling errors and analytical errors are therefore less serious in records of change of titratable acidity than in records of the total acidity. In using these records of change of titratable acidity it must be remembered that it is necessary to know that no change in the nature of the acid has occurred and that no change (or a known change) in the aluminium or ammonium content of the tissue has occurred.

Special analytical methods. (*a*) The method employed by Franzen and his co-workers(15, 17) is briefly as follows. The acids are precipitated as their lead salts by lead acetate. This precipitate is freed from protein and carbohydrate compounds by decomposing their lead compounds with CO_2 and washing. The acids are then set free from the purified precipitate by H_2S, and are then converted into their ethyl esters. The ethyl esters are then fractionally distilled *in vacuo*.

This effects a rough separation of the acids. The separated esters are then converted into the hydrazides of their acids, by shaking the ester with hydrazine hydrate in alcoholic solution, when the reaction

$$R.COOC_2H_5 + NH_2.NH_2 = R.CO.NH.NH_2 + C_2H_5OH$$
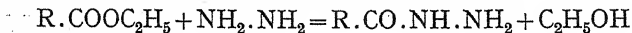
occurs. The hydrazides of the commoner acids are relatively easy to separate from each other, and the acids may be identified by the melting-points of these hydrazides. The method has not been developed as a strictly quantitative one, but it gives the most satis-

factory estimate available of the quantities of the acids which are present only in traces. Details are given in several papers by Franzen (15–17).

(b) *Polarimetric methods.* The commonest optically active plant acids are malic and tartaric, but since both of these acids are present in both *d*- and *l*- forms polarimetric methods can only be used in conjunction with other analytical methods to determine the relative quantities of the two isomers.

In estimating the excess of one of the active isomers it is usual to make use of Walden's discovery that uranium salts (UO_2NO_3, etc.) in the presence of NaOH or ammonia enormously increase the rotation of α-hydroxy acids. This is supposed to be due to the formation of a compound in which uranium is attached to the α-hydroxy oxygen atom. In dilute solution the molecular rotation of *l*-malic acid is about $-2\cdot5°$, while that of the uranium compound in presence of ammonia is $-436°$. Tartaric, lactic, and other hydroxy acids behave in a similar manner.

Some molybdenum and tungsten compounds also enhance the optical activity of α-hydroxy acids, and the method developed by Auerbach and Krüger has been used in investigations of the malic acid metabolism of muscle. References are given by Needham (31).

(c) Succinic acid, unlike malic, tartaric, citric, oxalic, and the other commoner plant acids is not readily oxidised by boiling permanganate solutions, consequently when these acids are removed by oxidation the succinic acid can be extracted from the residue with ether, and can then be precipitated and weighed as silver succinate (31, 1).

(d) The most satisfactory special methods for the estimation of citric acid depend on the fact that warming it with sulphuric acid and $KMnO_4$ converts it into acetone-dicarboxylic acid with loss of CO_2. In the method developed by Kunz (28) the acetone-dicarboxylic acid so formed is converted by bromine into pentabromacetone which is insoluble, and from the weight of this the quantity of citric acid is obtained.

(e) Acids containing an active methylene group

$$(-CO.CH_2.CO-)$$

such as acetone-dicarboxylic acid, $COOH.CH_2.CO.CH_2.COOH$,

oxalacetic acid, $COOH.CH_2.CO.COOH$,

acetoacetic acid, $CH_3.CO.CH_2.COOH$,

and malonic acid, $COOH.CH_2.COOH$

react with diazo-compounds to give hydrazones, which, in general, are very sparingly soluble.

For example, acetone-dicarboxylic acid reacts with benzene-diazonium chloride thus:

$$
\begin{array}{l}
CH_2.COOH \\
| \\
CO \\
| \\
CH_2.COOH
\end{array}
+ 2C_6H_5.N:N.Cl =
\begin{array}{l}
CH:N.NH.C_6H_5 \\
| \\
CO \\
| \\
CH:N.NH.C_6H_5
\end{array}
+ 2HCl + 2CO_2
$$
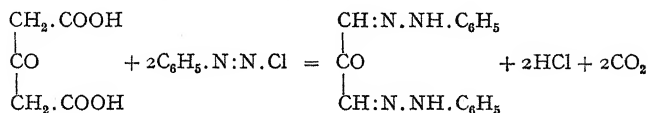
and the insoluble phenylhydrazone of mesoxalic aldehyde is formed. The method has been used for the detection of acetone-dicarboxylic and malonic acids among the products of metabolism of mould fungi[12, 46], but has not been developed as a quantitative method.

(*f*) Denigès' well-known tests deserve mention. The reagent used is a solution of mercuric sulphate + sulphuric acid in water. On warming it with a solution containing citric acid (not above 50° C.) and adding potassium permanganate solution drop by drop, the citric acid is oxidised and the basic mercuric salt of acetone-dicar-boxylic acid is precipitated as a white crystalline precipitate. This test, like most precipitation tests, is not absolutely critical, as other substances also give precipitates. Under certain conditions a double mercuric oxalate salt is precipitated (Bernhauer and Siebenäuger[10]). Acetone also yields a precipitate with the hot reagent. A modification of the same test is sometimes used as a test for malic acid; mercuric acetate + acetic acid is used instead of the sulphates, and, under the same conditions as for the citric acid test, malic acid yields a precipitate of a mercuric oxalacetate compound.

CHEMICAL PROPERTIES OF THE PLANT ACIDS WHICH HAVE
INFLUENCED BIOLOGICAL HYPOTHESES

(1) *Oxalic acid. Methods of formation.* (*a*) Sodium oxalate is formed by the direct combination of sodium with CO_2 at high temperatures: corresponding with this fact is the well-known theory of photo-synthesis of Liebig, which more recently has received the support of Baur[6] and others. Direct evidence in favour of the view that the oxalic acid in green plants is formed by reduction of CO_2 is not available.

(*b*) Oxalic acid is readily formed *in vitro* by the oxidation of many aliphatic and other compounds, such as carbohydrates, malic, tartaric acids among others, and alcohol, acetic, glycollic and

glyoxylic acids. The oxidation of acetic acid presumably follows the course:

$$CH_3COOH \rightarrow CH_2OH.COOH \rightarrow CH:O.COOH \rightarrow COOH.COOH$$
Acetic acid   Glycollic acid   Glyoxylic acid   Oxalic acid
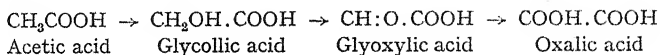
Probably glyoxylic acid is almost always the direct precursor of oxalic acid, when it is formed *in vitro* by oxidation of various aliphatic compounds.

The ready conversion of a great number of products into oxalic acid *in vitro* has suggested that this acid may be formed in plants in many different processes, but it has so far been found impossible to obtain direct evidence of the nature of the precursors of oxalic acid. In the special case of the mould fungi it is possible to obtain some experimental evidence.

Many strains of *Aspergillus* produce large quantities of oxalic acid when grown on such substrates as carbohydrate, malic acid, etc.: they also produce oxalic acid when supplied with acetic acid. It has consequently been generally assumed that the respiratory processes of these moulds follow a course such as the following:

Carbohydrate $\rightarrow$ X $\rightarrow$ acetic acid $\rightarrow$ glycollic acid $\rightarrow$
glyoxylic acid $\rightarrow$ oxalic acid $\rightarrow$ CO_2
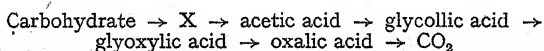
(cf. for example refs. (11, 12, 30); and Part III of this communication for further details). The accumulation of oxalic acid is readily explained on the assumption that the velocity of its formation exceeds that of its decomposition, and, similarly, the lack of accumulation of glycollic and glyoxylic acids must be due to the velocity constants of formation of these acids being less than those of their decomposition.

On the basis of this hypothesis one would therefore expect that oxalic acid would be more rapidly produced from glyoxylic and glycollic acids than from acetic acid. Now it is experimentally found that certain strains of *Aspergillus* which produce oxalic acid rapidly from acetic acid do not produce oxalic acid when supplied with glycollic acid and glyoxylic acid although they cause these latter acids to disappear ((32), also the author's unpublished results discussed in the last section of this paper). It seems clear that in this case the oxalic acid is not formed by this postulated step-by-step oxidation process, and consequently there is no certainty that such oxidation of glycollic and glyoxylic acids ever occurs in plant tissues.

Wetzel ((48), p. 495) also concludes that oxidation of such com-

pounds as acetaldehyde and acetic acid is unlikely to take place by any reaction other than

$$\begin{array}{c} CH_3.COOH \\ + \\ CH_3.COOH \end{array} - 2H = \begin{array}{c} CH_2.COOH \\ | \\ CH_2.COOH \end{array}$$
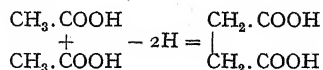
No evidence in favour of this view is, however, cited by Wetzel. In view of the facts briefly alluded to, it is necessary to consider reactions by which oxalic acid might be produced other than those in which glyoxylic acid is an essential intermediate product, and in which direct oxidation of acetic acid or aldehyde is involved.

(c) The only reaction which seems likely to be of significance is the hydrolysis of oxalacetic acid. Oxalacetic and acetoacetic acids in the form of their esters undergo two types of hydrolysis known as "acid hydrolysis" and "ketonic hydrolysis" respectively. The course of these reactions is

(1) $\begin{array}{c} CH_3.CO.CH_2.COOH \\ + HO.H \end{array} = 2CH_3COOH,$

(2) $\begin{array}{c} COOH.CO.CH_2.COOH \\ + HO.H \end{array} = COOH.COOH + CH_3COOH,$

(3) $\begin{array}{c} CH_3.CO.CH_2.COOH \\ + H{-}OH \end{array} = CH_3.CO.CH_3 + CO_2 + H_2O,$

(4) $\begin{array}{c} COOH.CO.CH_2.COOH \\ + H{-}OH \end{array} = COOH.CO.CH_3 + CO_2 + H_2O.$
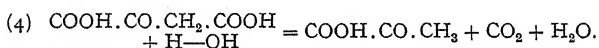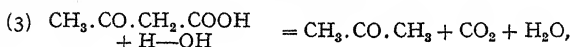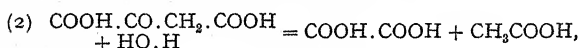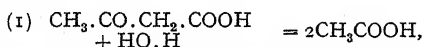
The ordinary enzymic decomposition of oxalacetic acid is usually supposed to be brought about by carboxylase, and results in the formation of pyruvic acid. Further action of carboxylase converts the pyruvic acid into CO_2 and acetaldehyde. It seems likely, however, that the first stage in the process (the conversion of oxalacetic to pyruvic acid) is independent of the action of the enzyme carboxylase, which acts on the group —CO.COOH, and that it corresponds to the ketonic hydrolysis of aceto-acetic ester (reactions 3 and 4). This hydrolysis slowly proceeds in aqueous solutions of the free acids. The "acid hydrolysis" occurs in alkaline alcoholic solutions of the esters of the acids.

An enzyme system capable of bringing about the acid hydrolysis of oxalacetic acid is not yet known, but, if present, it could account for the formation of oxalic acid in plants which are known not to convert glyoxylic and glycollic acids into oxalic acid.

The disappearance of oxalic acid from plant tissues. Oxalic acid can be reduced *in vitro* to glyoxylic acid, oxidised to CO_2, or converted to formic acid, and apart from reactions with nitrogen compounds or the formation of esters, other processes are not possible for its

disappearance *in vivo*. Upholders of the hypothesis that it is an intermediate product in the photosynthetic reduction of CO_2 consider that the oxalic acid is reduced to glycollic acid which is further converted to sugars. The view is more widely held at present that the acid is oxidised to CO_2 and water.

The existence of an enzyme has been reported which causes decomposition of the acid (Bassalik[4], Staehelin[41]). The enzyme is destroyed by heat at a remarkably slow rate. Oxygen is said to be necessary for its activity and it is described as an oxidase. The quantity of CO_2 formed, however, when a given quantity of oxalate disappears is too small to account for the observed loss of acid by oxidation, so the exact nature of the process is at present unknown.

(2) *Malic and the related acids.* Malic acid is mono-hydroxy-succinic acid; both the laevo- and dextro-rotatory forms of this acid occur in plants, but in fruits such as the apple the laevo- form greatly predominates. In the leaves of the succulent plants, in *Rheum*, and probably in many others, the quantities of the two forms are nearly equal. Data are at present not available of the effects of varying natural or experimental conditions on the relative quantities of the two isomers.

The malic acid found in members of the Crassulaceae is frequently reported to be isomalic acid, $CH_2OH.CH(COOH)_2$. The reason for this is that malic acid extracted from the Crassulaceae was found by Mayer (29, 30) to give salts differing in certain respects from ordinary malates. Aberson (2) reinvestigated the behaviour of this acid and also noted differences in its behaviour from common malic acid, notably that the crassulaic acid gives rise on distillation *in vacuo* to malide, whereas the ordinary acid gives rise to a mixture of maleic and fumaric acids. Aberson showed that the crassulaic acid was a hydroxy-succinic acid, $COOH.CH_2.CHOH.COOH$[1], and not isomalic (hydroxy-methylmalonic) acid. Franzen and Ostertag (18) have more recently shown that the sole difference between the crassulaic acid and ordinary malic acid is that the former is a mixture of the ordinary acid with small amounts of the anhydride form, malyl-malic anhydride

$$COOH.CH.CH_2.CO.O$$
$$O.CO.CH_2.CH.COOH,$$
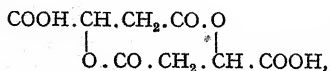
which they isolated as its diethyl ester. It is not clear that the anhydride is present in the living tissues, as it is quite possible that it is formed in the processes of extracting the malic acid from them.

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[1] Theoretically only two forms of hydroxy-succinic acid are possible, *d*- and *l*-malic acids.

When malic acid is heated it loses water and a number of products are formed according to the conditions under which the heating is carried out. The first products are malyl-malic acid,

$$COOH.CH_2.CH(COOH).O.CO.CH_2.CHOH.COOH,$$
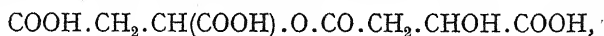
and malide. On further heating one or two molecules of water are lost from each molecule of acid with the formation of maleic anhydride and fumaric acid.

H.C.COOH	H.C.COOH	H.C.CO
‖	‖	‖ $>$O
COOH.C.H	H.C.COOH	H.C.CO
Fumaric acid	Maleic acid	Maleic anhydride
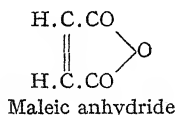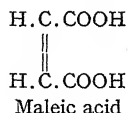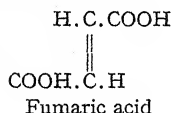
Maleic acid is not found in living tissues, but fumaric acid is widely distributed. An enzyme, fumarase, converts fumaric acid into an equilibrium mixture of fumaric and *l*-malic acids, and the same equilibrium mixture is attained when the enzyme acts on *l*-malic acid alone. Fumarase is widely distributed in animal tissues and bacteria (Woolf (50)), and is presumably present in some mould fungi, since Challenger and Klein (13) have shown that *Aspergillus niger* converts fumaric acid into *l*-malic acid; *d*-malic acid is not formed, or at all events was not detected. It is at present uncertain whether the *d*-malic acid in plants is accompanied by a *d*-fumarase, or whether its fate differs from that of *l*-malic acid.

Malic acid is readily oxidised *in vitro*, strong oxidising agents give oxalic acid and finally CO_2; gentle oxidation, by hydrogen peroxide for example, gives oxalacetic acid. It is accordingly believed by many that the disappearance of malic acid from plant tissues involves this process, and the changes which are known to occur in resting bacteria lend some support to this view, though they suggest that the conversion is not effected by direct oxidation of the malic acid.

Resting bacteria in the absence of oxygen but in presence of a suitable hydrogen acceptor such as methylene blue can bring about the dehydrogenation of succinic acid to fumaric acid

$$COOH.CH_2.CH_2.COOH + meth.\ blue \rightleftharpoons$$
$$COOH.CH:CH.COOH + leuco\text{-}meth.\ blue,$$
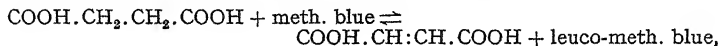
but they do not dehydrogenate malic acid to oxalacetic acid, nor do they dehydrogenate fumaric acid. They do, however, convert fumaric acid in the presence of oxygen to pyruvic acid, and it is almost certain that oxalacetic acid is formed first, and then loses CO_2 in the usual way. The reactions are included in Table II.

TABLE II

Biological inter-relationships of the malic acid group

$$
\begin{array}{ccc}
\text{COOH} & & \text{COOH} \\
| & & | \\
\text{CH}_2 & \text{CH.COOH} & \text{CH.OH} \\
| \quad +2\text{H} & \parallel \quad\quad +\text{H}_2\text{O} & | \\
\text{CH}_2 \rightleftharpoons & \text{COOH.CH} \rightleftharpoons & \text{CH}_2 \\
| & & | \\
\text{COOH} & & \text{COOH}
\end{array}
$$

Succinic acid   Fumaric acid   Malic acid

$$\downarrow\uparrow \; ?$$

$$\downarrow \; +\text{O}$$

$$
\begin{array}{cc}
\text{CH.COOH} & \text{COOH} \\
\parallel \quad\quad \rightleftharpoons & | \\
\text{COOH.C.OH} \quad ? & \text{CH.OH} \\
 & | \\
 & \text{CH.OH} \\
 & | \\
 & \text{COOH}
\end{array}
$$

Hydroxy-fumaric acid   Tartaric acid

$$\downarrow\uparrow \quad\quad\quad\quad \downarrow\uparrow \; ?$$

$$
\begin{array}{c}
\text{C(OH).COOH} \\
\parallel \\
\text{C(OH).COOH}
\end{array}
$$

Dihydroxy-maleic acid

$$
\begin{array}{ccccc}
\text{CO}_2 & & \text{COOH} & & \downarrow\uparrow \; ? \\
+ & & | & & \\
\text{CH}_3 & & \text{CH}_2 & & \text{CH.COOH} \\
| & \leftarrow & | \quad \rightleftharpoons & & \parallel \\
\text{CO} & & \text{C}=\text{O} & & \text{C(OH).COOH} \\
| & & | & & \\
\text{COOH} & & \text{COOH} & & \\
\end{array}
$$

Pyruvic acid   Oxalacetic acid   Hydroxy-maleic acid

$$\downarrow \quad\quad\quad\quad \downarrow \; +\text{H}_2\text{O}$$

$$
\begin{array}{cc}
\text{CH}_3 & \text{COOH} \\
| & | \\
\text{CH}=\text{O} & \text{CH}_3 \\
+ & + \\
\text{CO}_2 & \text{COOH} \\
 & | \\
 & \text{COOH}
\end{array}
$$

Acetaldehyde   Acetic + oxalic acid

It seems likely that the reactions given in this schema can occur in plant tissues, but evidence will be developed later which makes it doubtful if they represent the fate of more than a rather small fraction of the malic acid in plants. Incidentally, to complete the explanation of the reactions given above, one may mention that oxalacetic acid can exist in three forms; the keto- form which predominates in its salts, and two end-forms hydroxy-maleic acid which is formed when the acid is liberated from its salts by weak acids, and hydroxy-fumaric acid formed by the action of strong acids on hydroxy-maleic acid.

It has been known for a long time that the malic acid present in plant tissues disappears when they are illuminated, and attempts have been made to show that a photochemical reaction is responsible for this acid loss. Spoehr(40) showed that the acidity of expressed sap of *Opuntia* decreased when the sap was exposed to sunlight. This decrease was not due to the activity of an enzyme as Spoehr found that addition of the ash of the plant to pure malic acid solutions enabled this photodeacidification to take place at about the same rate as was obtained in the case of the expressed sap. This rate was, however, some thirty or forty times less than the rate at which the acid disappears from the intact living plant. Spoehr supposed that the first stage in the process was the splitting off of CO_2 from the carboxyl groups with the formation of alcohol:

$$COOH.CH_2.CHOH.COOH = CH_3.CH_2.OH + 2CO_2.$$
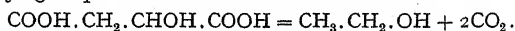
The other products besides alcohol, which are detectable, are supposed to arise by further oxidation of the alcohol; they include aldehyde, glycollic, glyoxylic, and oxalic acids, formaldehyde and formic acid among others. Decompositions of aqueous solutions of other hydroxy acids in sunlight in presence of certain salts are fairly well known.

Since the disappearance of acid in light in the living tissues is prevented by the action of narcotics (as was, indeed, well known before Spoehr's work, cf. Astruč(3)) it seems highly improbable that it is not intimately linked with the vital activities of the tissues. The matter is discussed later in dealing with the succulent plants.

This account may be completed by noting that the amino-acid corresponding to malic acid, aspartic acid, and its acid amide asparagin, $COOH.CHNH_2.CH_2.CO.NH_2$, are widely distributed often in considerable quantity. Asparagin is intimately connected with the protein metabolism; and this has suggested that the malic acid originates from protein rather than carbohydrate sources (cf. Kostytchev(25)). This by no means follows from the somewhat scanty evidence, nor is there any direct reason to suppose that because the nitrogen in the asparagin molecule is derived from protein that the carbon skeleton must also be derived from protein rather than from carbohydrate.

The difficult problems of the origin of malic acid in plants will be considered in connection with the behaviour of specific plant types.

(3) *Citric acid*. The branched chain structure of citric acid,

$$COOH.CH_2.C(OH).CH_2.COOH,$$
$$|$$
$$COOH$$
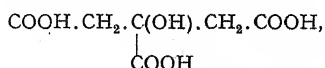
has given rise to many hypotheses as to its origin in plants. Euler in 1903 [14] suggested that it was formed by the condensation of acetaldehyde and oxidation of the product thus:

$$
\begin{array}{lll}
H.CH_2.CH:O & CH_2.CH:O & CH_2.COOH \\
\quad\quad\quad\quad\quad\quad | & | \\
+ O:CH.CH_3 \;=\; H_2O + & CH.CH_3 & \rightarrow \quad C(OH).COOH \\
\quad\quad\quad\quad\quad\quad | & | \\
+ H.CH_2.CH:O & CH_2.CH:O & CH_2.COOH
\end{array}
$$
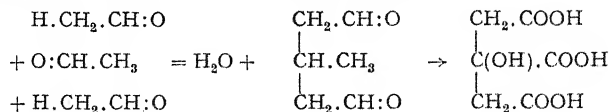
There is actually no experimental basis for believing that these reactions could take place.

The same remarks apply also to Butkevitsch's view that the conversion of sugars to citric acid by mould fungi takes place by condensation involving formation of a 5-carbon ring thus:

$$
\begin{array}{lll}
CHOH\!-\!CHOH & CHOH\!-\!CHOH & COOH\quad COOH \\
| \quad\quad\quad | & | \quad\quad\quad | & | \quad\quad\quad | \\
CH:O \quad CHOH \rightarrow & CHOH \quad CHOH \rightarrow & CH_2 \quad CH_2 \\
\quad\backslash\quad\diagup & \quad\backslash\quad\diagup & \quad\backslash\quad\diagup \\
CHOH & COH & COH \\
| & | & | \\
CH_2OH & CH_2OH & COOH
\end{array}
$$
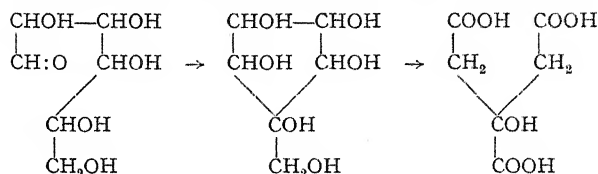
The hypothesis of Franzen and Schmitt [23] is based on the fact that diketones undergo the following reaction, known as the benzilic acid transformation, in weakly alkaline solutions:

$$
R.CO.CO.R' + H_2O = \genfrac{}{}{0pt}{}{R}{R'}\!\!\diagdown C(OH).COOH ;
$$
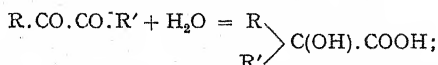
thus $\beta:\gamma$-diketoadipic acid was shown by Franzen and Schmitt to be transformed into citric acid. They supposed that glucose was oxidised to gluconic and then to saccharic acid, and that the latter yielded diketo-adipic acid by loss of water, so that the complete sequence of reactions is as follows:

$$
\begin{array}{llllll}
CHO & COOH & COOH & COOH & COOH & COOH \\
| & | & | & | & | & | \\
CHOH & CHOH & CHOH & CH & CH_2 & CH_2 \\
| & | & | & \| & | & | \\
CHOH & CHOH & CHOH & C.OH & CO & C(OH).COOH \\
| \;\rightarrow & | \;\rightarrow & | \;\rightarrow & \| \;\rightarrow & | \;\rightarrow & | \\
CHOH & CHOH & CHOH & C.OH & CO & CH_2 \\
| & | & | & \| & | & | \\
CHOH & CHOH & CHOH & CH & CH_2 & COOH \\
| & | & | & | & | \\
CH_2OH & CH_2OH & COOH & COOH & COOH \\
\text{Glucose} & \text{Gluconic} & \text{Saccharic} & \text{Diketo-} & \text{Diketo-} & \text{Citric acid} \\
& \text{acid} & \text{acid} & \text{adipic} & \text{adipic} \\
& & & \text{acid (enol)} & \text{acid (keto)}
\end{array}
$$
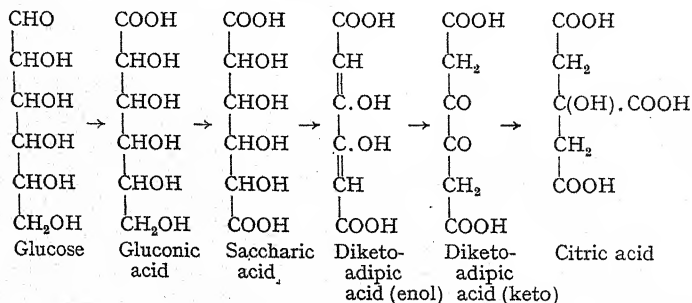
Although the hypothesis is reasonable enough from the purely chemical point of view, recent work on the citric acid production by

moulds has suggested that the citric acid may be formed from acetic acid thus:

$$\begin{array}{cccc} CH_3.COOH & CH_2.COOH & CH_2.COOH & CH_2COOH \\ & \overset{-2H}{\to} \ \mid & \to \ \mid & \mid \\ +\,CH_3.COOH & CH_2.COOH & CHOH.COOH & \overset{-2H}{\to} \ C(OH).COOH \\ & & +\,CH_3.COOH & CH_2COOH \end{array}$$
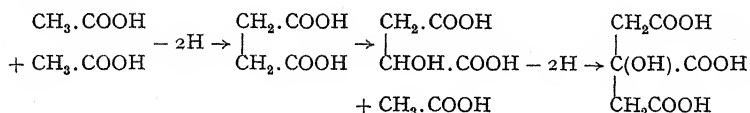
It should be mentioned that with the exception of the oxidation of succinic to malic acid these reactions cannot be imitated *in vitro*. The hypothesis is of interest in that it emphasises the close relationship, which is actually found, between malic and citric acid.

Citric acid is readily oxidised *in vitro* to acetone-dicarboxylic acid, and it is therefore believed by most investigators that its disappearance from living tissues follows this course (cf. for example, Bernhauer and Siebenäuger[11]). Walker, Subramaniam and Challenger[46, 12] succeeded in detecting acetone-dicarboxylic acid, and also acetone, malonic and acetic acids in cultures of *Aspergillus niger* grown on solutions of ammonium citrate and citric acid. They therefore suppose that the disappearance of citric acid from tissues involves the reactions

$$COOH.CH_2.COH(COOH).CH_2.COOH + O = COOH.CH_2.CO.CH_2.COOH \\ + H_2O + CO_2$$

$$COOH.CH_2.CO.CH_2.COOH + H.OH = COOH.CH_3 + COOH.CH_2.COOH$$

$$COOH.CH_2.CO.CH_2.COOH = CH_3.CO.CH_3 + 2CO_2$$
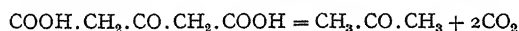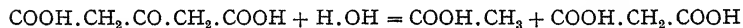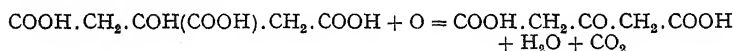
These reactions can evidently take place in the mould fungi, but they may account for the fate of only a very small proportion of the citric acid which is converted into other substances, as evidence which will be discussed later indicates.

FACTORS AFFECTING THE ACID CONTENT OF PLANT TISSUES

The striking changes in the acidity of plant tissues which occur under natural conditions have rightly induced investigators to consider that the organic acids play some important rôle in metabolism. These changes are associated with the normal diurnal and seasonal fluctuations in light intensity and temperature.

The effects of other external conditions artificially controlled, such as continued starvation in darkness, and exposure to gas mixtures other than air, have been examined in certain cases and have yielded results which have greatly influenced our valuation of the many hypotheses which purport to explain the rôle of the plant acids in metabolism. The results of such experimental work can

however be more conveniently considered in connection with our accounts of the hypotheses which they have evoked or condemned. This section, therefore, deals only with diurnal and seasonal fluctuations in acidity and their distribution throughout the plant kingdom.

The first known case of fluctuation in the acidity of a plant tissue was the observation that the sap of *Bryophyllum* (one of the Crassulaceae) increased markedly in acidity during the night and again decreased during the day. All the Crassulaceae behave in a similar manner, and it has since been shown that almost all succulent plants are similar; the noteworthy exceptions belong to the order Centrospermae (*Mesembryanthemum, Portulaca,* and some halophytes). Many non-succulent plants show similar behaviour. The phenomenon is therefore widespread, but, as the data collected here show, a much more extended survey of the plant kingdom is desirable.

Crassulaceae. Members of this order contain malic acid with traces of succinic, oxalic and citric acids. The commonly found statement that the acid is isomalic has been shown to be erroneous (cf. p. 54). The nature and extent of the diurnal fluctuation in acidity are indicated by the figures in Table III, which records the titratable acid contents of leaves of *Bryophyllum calycinum* at different hours of the day. The data are taken from Kraus(26, 27), and refer to mature leaves which remained attached to the plant which was growing under natural conditions.

TABLE III

Titratable acidity as mg. equiv. acid per 100 grm. fresh weight

Hour	Acidity	Hour	Acidity	Hour	Acidity
6 a.m.	12·25	4 p.m.	2·0	2 a.m.	8·30
8 ,,	10·0	6 ,,	1·38	4 ,,	12·0
10 ,,	10·50	8 ,,	2·25	6 ,,	11·8
12 noon	8·75	10 ,,	4·25	8 ,,	12·0
2 p.m.	4·63	12 midnight	6·75		

Kraus showed that this fluctuation in acidity was associated with a fluctuation in the carbohydrate content in the opposite direction (maximum sugar content in the evening, minimum in the morning), and he showed moreover that the change in titratable acidity was not connected with neutralisation of the acid by bases. It seems therefore clear that the nightly increase in acidity is brought about by the formation of malic acid from sugar. The acid formed does not all remain in the form of free acid but part dissociates so that an equilibrium mixture of acid and its ions is obtained as indicated in the introductory discussion. It is clearly not correct to state that the

new formed acid is converted into the acid salt (cf. Wolf (49)) though under certain conditions a large fraction of the acid will be changed to the form of univalent ions.

Similar results to these recorded for *Bryophyllum* in Table III are also recorded in Fig. 2 for *Sedum praealtum*[1], *Bryophyllum* and *Opuntia*. The time of day is indicated on the horizontal axis. In this case the *Bryophyllum* leaves were attached, and the *Sedum* leaves
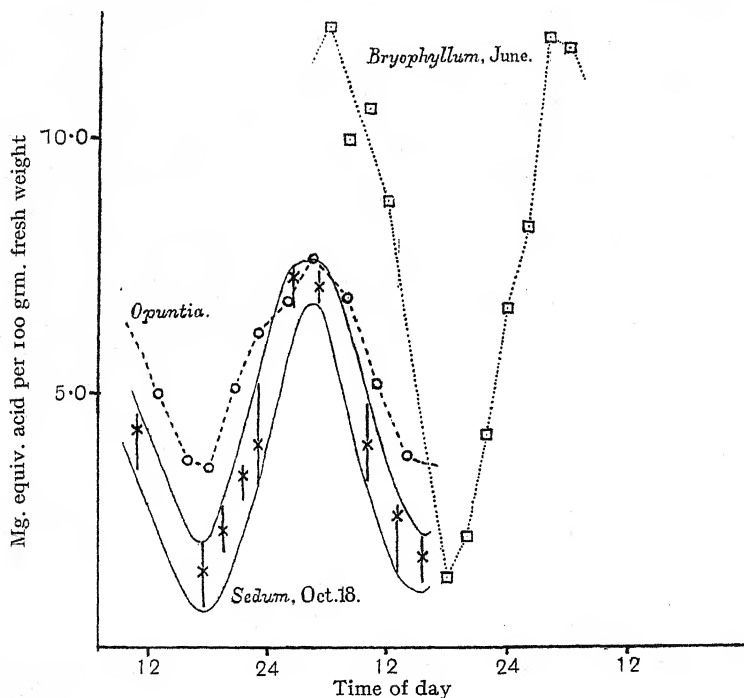
Fig. 2. Change of titratable acidity during the day of leaves of *Sedum* (9), *Bryophyllum* (27) and joints of *Opuntia* (33).

detached from the stems, the *Opuntia* joints were also detached and all were left under otherwise natural conditions. All the material behaved, it will be noted, in essentially the same manner.

It is important to be clear that the change in titratable acidity is not due to neutralisation of acid by ammonia in view of Bendrat's results and criticisms (see p. 41). Unless ammonia is formed it is

[1] The vertical lines indicate roughly the sampling errors. The top of the line gives the highest acidity of a group of four analyses, and the bottom the lowest: the cross gives the average acidity.

clear that the non-titratable acidity will not fluctuate during the night and day. Wolf's results make it clear that the changes in ammonium content throughout the day are insignificant as a source of error in the acidity determinations. These results also show that the extent of the diurnal fluctuation in the nitrogenous constituents of the leaves are so small as to exclude the possibility that the malic acid is derived from them. These results are given in Table IV; the quantities are given as mg. or mg. equivalents per 100 grm. fresh weight. Since the common amides (asparagin, glutaminic acid) have two nitrogen atoms per molecule the quantity "2 × amide N" equals asparagin, etc. nitrogen. The soluble nitrogen minus ammonia nitrogen minus "2 × amide nitrogen" consists chiefly of amino-acid nitrogen. For conciseness the German term "Rest-N" is used for this quantity.

TABLE IV

Diurnal nitrogen and acid metabolism. Quantities are expressed per 100 grm. fresh weight

		Bryophyllum calycinum		Bryophyllum proliferum	
		5.30 p.m.	9.00 a.m.	5.00 p.m.	9.00 a.m.
Protein-N	mg.	168·00	163·00	240·00	233·00
	mg. equiv.	12·00	11·60	17·10	16·60
NH_3-N	mg.	1·00	0·55	0·68	0·57
	mg. equiv.	0·07	0·04	0·05	0·04
2 × amide-N	mg.	5·35	6·80	4·85	6·90
	mg. equiv.	0·38	0·49	0·35	0·49
Rest-N	mg.	16·15	20·15	19·47	28·52
	mg. equiv.	1·15	1·44	1·39	2·04
Total malate	mg. equiv.	13·10	26·00	14·40	24·00

It will be noted that there is no loss of protein + amino acid during the night corresponding with the gain in acidity. The amide (probably asparagin) content rises when the malic acid content rises, and the ammonia content decreases, which would be expected if the equilibrium occurred:

$$\text{malic acid} + \text{ammonia} \rightleftharpoons \text{asparagin}.$$
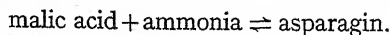
At the most, however, 0·2–0·3 per cent. of the titratable acid is involved in this process in the cases recorded.

Wolf also showed that the decrease in sugar content during the night was more than great enough to account for the acid production in almost all cases. For example, for *Bryophyllum calycinum*, the

changes in sugar and acid content between 5 p.m. and 9 a.m. are given as:

TABLE V

Temperature	Loss of reducing sugar as mg. mol. per 1 grm. dry weight	Gain in malic acid as mg. mol. per 1 grm. dry weight
8°	0·32	0·21
20°	0·50	0·50
35°	0·43	0·22
10°	0·26	0·42
21°	0·56	1·10
37°	0·46	0·24

The present writer's results, which are discussed later, also indicate that the formation of one mol. of malic acid is associated with the disappearance from the tissue of at least one mol. of reducing sugar.

The gradual ageing of the leaves effects considerable changes in their acid metabolism, of which the most striking is the gradual neutralisation of titratable acid by bases absorbed from the soil. The type of results usually found is indicated by those of Astruč given in Table VI.

TABLE VI

Acidity of leaves of Sempervivum tectorum.
Mg. equiv. per 100 grm. fresh weight

Position of leaf	Titratable acidity		Non-titratable acidity	
	Morning	Evening	Morning	Evening
1 (tip)	3·6	4·4	5·0	7·4
4	4·4	3·8	7·4	8·2
6	5·4	2·6	14·4	14·4
8	6·0	2·0	15·4	14·4
10	8·8	2·0	22·6	24·2
12 (base)	7·5	1·8	31·4	30·4

Note that there is no diurnal fluctuation in non-titratable acid (alkalinity of ash), and that the fluctuation in titratable acidity is not noticeable in the youngest leaves. It decreases slightly as the leaves become senescent. Bendrat also records very slight fluctuation in the young leaves and notes also greater acidity at night than in the morning in young leaves. Unfortunately both Astruč and Bendrat's conclusions are based on isolated pairs of analyses, so no complete record of the behaviour of very young leaves is available.

The extent of the diurnal fluctuation differs at different seasons. Kraus gives these data for *Bryophyllum*: Feb. 20, morning 3·75 mg. equiv. per cent., evening 1·2 mg. equiv. per cent.; June 27, morning

13·8 mg. equiv. per cent., evening 1·1 mg. equiv. per cent. More complete data illustrating the same phenomenon are given in Fig. 3. The two curves illustrate the change in the values of the maximum and minimum diurnal acidities respectively throughout the seasons. Each recorded value of the acidity is shown as a vertical line which indicates the highest and lowest of four estimations and each is taken from a curve of the type given in Fig. 2. They do not represent the
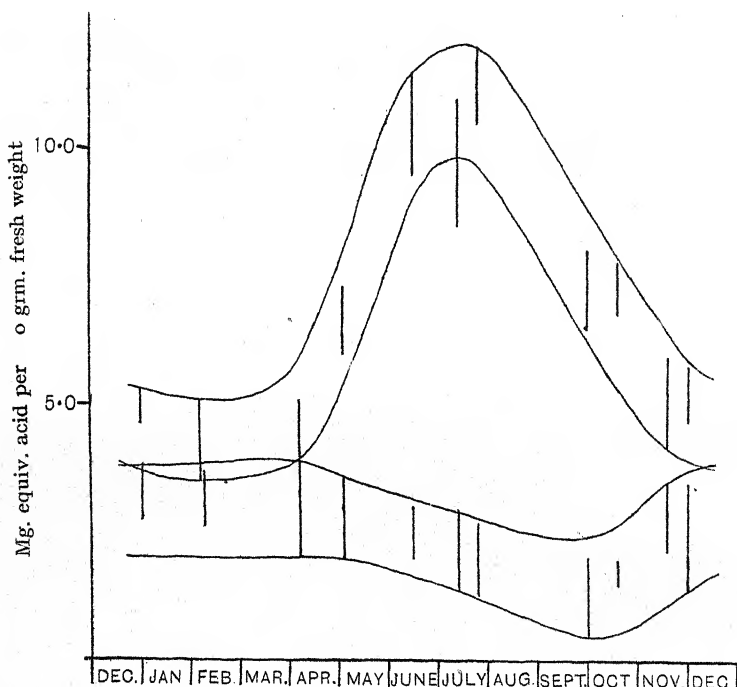
Fig. 3. Maximal diurnal titratable acidity and minimal diurnal titratable acidity at different seasons of the year. *Sedum praealtum* (9).

acidities taken at arbitrary hours in the morning and evening. The diurnal fluctuations in the latitude of Dublin (where these records were made) are practically non-existent for at least four months in winter.

In addition to the effects of age and season, soil conditions evidently have very marked effects on the acidity of succulent and probably other plants (cf. (34)). The writer (9) found, for example, that two plants of *Crassula lactea* of the same age, which were cuttings from the same parent plant had titratable acidities of 8·3

and 2·2 mg. equiv. per 100 grm. fresh weight at their diurnal maximum acidity in June. The plants were grown in different soils, but the exact cause of the very striking difference is still uncertain. It was at first thought that low acidity of the one plant was due to the more extensive neutralisation of the acid by bases absorbed from the soil, as this plant was grown in relatively alkaline soil (pH 9), whereas the other was grown in almost neutral soil (pH 6·8); further, the total malate was roughly equal in both plants. Ruhland and Wetzel(34) show, however, that rich nitrogen manuring causes a considerable rise in acidity in the case of *Begonia*, and it is consequently possible that different nitrogen supplies rather than different soil acidities brought about the changes observed in *Crassula lactea* and also in *Sedum praealtum*. The changes in respiration correlated with these changes in acidity are dealt with later. These remarks will make it clear that it is most necessary to know the exact conditions both of soil and climate under which succulent plant material is grown when comparisons are being made.

Cactaceae. Members of this order show the typical photo-deacidification which is also characteristic of the Crassulaceae. Conversion of carbohydrate to malic acid proceeds by night, and the reverse process, associated with photosynthesis, occurs by day. Typical results are illustrated by the curve relating to *Opuntia* (Richards(33)) which is given in Fig. 2.

Other succulent plants. Unfortunately the only data available consist of isolated pairs of analyses, one taken in the evening and a second on the following morning in general indicating a higher titratable acidity. The results of a number of such pairs of results are given in Table VII. It will be seen that the behaviour of *Mesembryanthemum* and *Portulaca* is different from that of the other succulents which resemble the Crassulaceae.

These genera, as noted before, are rather rich in oxalic acid, and as the leaves age the ratio oxalic:malic acid rises. The irregularity of the behaviour may be connected with different oxalic:malic acid ratios, and differences in the fates of the two acids during the day. It is perhaps significant that Thoday and Evans(44) have observed a differentiation of the tissues into oxalate and malate containing cells. Evidently, even if the metabolism of the latter is of the typical crassulacean type, it will be masked by the behaviour of the oxalate cells, if the mode of analysis is the grinding up of the entire tissue.

Non-succulent plants. The data are inadequate but one can state with some confidence that the typical crassulacean metabolism which

is characteristic of succulents is widely distributed also among non-succulent plants. Two families, Orchidaceae and Bromeliaceae, especially exhibit diurnal fluctuations of large magnitude amongst almost all genera.

Table VII shows that a number of other non-succulent plants also show diurnal periodicity in acidity, the phenomenon is therefore widely distributed throughout the plant kingdom.

TABLE VII

Percentage changes in titratable acidity during the night
(increase shown as +)

Plant	% change	Author
Phoenix dactylifera	+ 44	Warburg
Billbergia zebrina	+ 86	,,
Nidularia mayendorfii	+ 95	,,
Hoplophytum grande	+ 286	,,
Tillandsia biflora	+ 74	,,
Tradescantia discolor	+ 36	,,
Aloe arborescens	+ 2	Hempel
,, cymbaefolia	+ 340	,,
Haworthia turgida	+ 80	Kraus
Sanseveria fasciata	+ 30	Warburg
Convallaria majalis	+ 2	,,
Clivia nobilis	+ 22	,,
Pancratium sp.	+ 22	,,
Cypripedilum villosum	− 70	Bendrat
Cattleya sp.	+ 133	Warburg
Oncidium sp.	+ 180	,,
,, sphacelatum	+ 79	Bendrat
Vanilla planifolia	+ 45	,,
Peperomia sp.	+ 40	Warburg
Ficus elastica	− 3	,,
Hakea laurina	+ 12	,,
Polygonum platycodon	+ 3	,,
Rumex obtusifolius	+ 25	,,
Rheum sativum	− 9	Steinmann
Mesembryanthemum linguaeforme	− 3	Warburg
,, ,,	− 60	Kraus
,, ,,	+ 14	,,
,, ,,	+ 35	Hempel
,, anthelminticum	+ 58	Warburg
Portulaca grandiflora	− 18	,,
Laurus nobilis	+ 15	,,
Crassula arborescens	+ 20	Kraus
Bryophyllum calycinum	+ 200	,,
	+ 1260	,,
Saxifraga elatior	− 15	Warburg
Vicia faba	+ 13	Kraus
Geranium pyrenaicum	+ 10	,,
,, pratense	+ 700	,,
Croton magnum	+ 64	,,
Begonia warczewiczii	0	Warburg
,, semperflorens	+ 80	Ruhland & Wetzel
Hedera helix	− 3	Warburg
Eryngium spinosissimum	+ 8	,,
Kleinia articulata	0	,,
,,	+ 30	,,
,,	+ 440	Thoday & Evans

Two non-succulent plants have been more fully investigated and require special mention. The results obtained indicate that some caution must be exercised in interpreting such results as are collected above.

Rheum sativum (common rhubarb). Steinmann showed, see Table VII, that there is a slight gain in titratable acidity during the day, and this gain is increased if the main veins of the leaf are cut, thus hindering transport. He concluded that the acid was formed during photosynthesis. The fact that rise in temperature accelerates respiratory CO_2 output, but causes the acid to decrease in quantity, further supported his view, that the acid was formed in photosynthesis and not respiration, apparently. The same may be said of Astruč's observations that the green parts of variegated leaves of *Pelargonium* and *Acer* contain about five times as much titratable acid as the neighbouring white parts.

It may be necessary to modify these conclusions in view of Ruhland and Wetzel's subsequent work (35, 36). They have shown that about half the total acid is present as ammonium salt, but they do not state whether diurnal fluctuations in ammonium content occur. Clearly if the ammonium content decreases by day, the titratable acidity would rise, without indicating any new formation of acid by photosynthesis or otherwise.

Ruhland and Wetzel deal chiefly with seasonal changes in acid content. About 60–70 per cent. of the nitrogen in the resting rhizome is in the form of amino acids, and on germination the amino acids disappear and ammonia and malic acid increase in quantity in the leaf-stalks and leaves: they may therefore be formed from the disappearing amino-acids. The molar concentration of ammonia is said to be equal to that of the *l*-malic acid, and this concentration equals half that of the inactive malate. Ruhland and Wetzel conclude from these facts that the *l*-malic is formed together with an equivalent of ammonia from the amino-acids, and that the *i*-malate does not arise *de novo* but is transported as such from the rhizome on germination. How this follows is not clear. It is obscured by the use of the term *i*-malic acid; in solutions, this "substance" is a mixture of equal numbers of molecules of dextro- and laevo-malic acid. So that actually the results quoted indicate that the molar concentration of ammonia equals that of the dextro- and not that of the laevo-malic acid as stated by Ruhland and Wetzel[1].

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[1] According to Ruhland and Wetzel,
[ammonia]: [*l*-malate]: [*i*-malate] = 1:1:2,
this means that [ammonia]: [*l*-malate]: [*d*-malate] = 1:2:1.

As the season advances the malic acid content decreases and the oxalic acid content increases. Ruhland and Wetzel state that there is an "Übergang von Äpfelsäure, und zwar *nur die inactive Form*, in Oxalsäure." According to them, only *i*-malic acid decreases in quantity, but not *l*-malic acid, and oxalic acid appears. This, however, surely must mean that laevo- and dextro-malate are being used up at the same rate notwithstanding the fact that the concentration of the laevo-acid is greater.

Finally, just before the commencement of yellowing of the leaves, only *l*-malate and oxalate are present, and during the yellowing process the oxalate is left in the leaves and the *l*-malate is transported down the stalks; but in the rhizome *i*-malic acid is found. The authors state that the *l*-malate is converted into *i*-malate; again, this must mean that for some obscure reason exactly half of the *l*-malate is converted into *d*-malate.

One awaits with much interest the publication of further information, indeed at this stage the significance of the results cannot be appreciated.

Begonia semperflorens. Ruhland and Wetzel(34) show that this plant is very rich in acids and their salts. About 20 per cent. of the dry weight is oxalate, 0·5 per cent. malate, and 0·3 per cent. other acids. Large quantities of ammonia are present as salts. The *p*H varies between 1 and 2. The titratable acidity rises during the night; whether this is a real rise or not is not clear, as the statement is made that the ammonia decreases in quantity during the night: on the other hand, the authors state that the bound acid remains constant in quantity throughout the 24 hours, which would not be possible if the ammonium content fluctuated.

Nitrogen manuring increases the oxalate content, and this effect is greater when the nitrogen is present as nitrates rather than ammonium salts. Similar results had been obtained previously by Benecke(8) using a variety of other plants, and they were related by him to the fact that assimilation of nitrate ions should liberate cations which fix the oxalate formed in metabolism, and so induce the formation of increased quantities of oxalate.

Summary

One may distinguish three groups of plants.

(*a*) Malic acid containing plants having the crassulacean type of acid metabolism (malic acid formed from carbohydrate in darkness and converted into it in the light). This group probably includes all

succulent plants with the exception of certain Centrospermae. Many non-succulent plants are of this type, the most noteworthy being the Orchidaceae and Bromeliaceae. Many plants containing malic acid in which diurnal periodicity has not been observed may of course have a similar type of metabolism with a fluctuation so small that it does not exceed the sampling errors.

(b) Malic acid plants in which the young parts contain chiefly malic acid, but in which oxalic acid appears and malic acid disappears as the plants become older. *Rheum* and *Mesembryanthemum* certainly belong to this group and probably many plants which are rich in oxalic acid are also of this type. *Rheum* and possibly other plants in this group are rich in ammonium. This fact emphasises the inter-relationship of the malic acid metabolism with the protein metabolism which will be discussed later, but does not alone indicate that the carbon part of proteins or amino-acids is the source from which malic acid is formed in plants of this type. Indeed malic acid produced from carbohydrate in the crassulacean manner might by its reaction with the ammonia cause a shift to the right-hand side of the equilibrium:

$$\text{Protein} \rightleftharpoons \text{carbon residue} + NH_3.$$
(or Amino-acid)
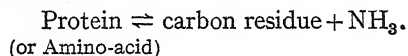
(c) Oxalic acid containing plants. *Begonia* and *Oxalis* are certainly to be regarded as members of this group. They should, perhaps, be regarded as a special case of group (b) in which the change malic acid $\rightarrow$ oxalic acid (whether occurring directly or indirectly) proceeds much more rapidly than in such types as *Rheum*.

(d) This summary would be incomplete without mention of the fleshy fruits, which are invariably rich in acids of the malic or citric acid groups. As ripening (senescence) of the fruit advances the acid disappears, but investigation of the acid metabolism of fruits has so far failed to throw light on the chemical mechanism or significance of the process. There is at present no reason to suppose that the chemical mechanisms involved in the acid metabolism of mature leaves differs from those of senescing organs such as fruits, but the nature of the available evidence does not justify the inclusion of a discussion here.

(e) Mould fungi produce organic acids and their behaviour has greatly influenced hypotheses regarding the acid metabolism of green plants. Special peculiarities in their nutrition makes a comparison of their metabolism with other plants in general terms impossible. The acid metabolism of the fungi is discussed in the last section of this paper.

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REVIEWS

Handbuch der Pflanzenanalyse. Herausgegeben von G. KLEIN. Julius
Springer, Wien, 1932. Zweiter Band. Spezielle Analyse, Erster
Teil. 10 × 7 in. S. xi + 973. RM. 96, gebunden RM. 99.

The first volume of this book has already been reviewed in the *New Phyto-
logist* of 1931. The present volume is constructed on similar lines and contains
seventeen articles by various authors dealing with the estimation of inorganic,
and some organic plant constituents. The parts are: common inorganic cations
and anions (A. Rippel); inorganic nitrogen (D. N. Prianischnikow and A. A.
Schmuck); analysis of the ash (W. Sutthoff); gas analysis (H. Kleinmann and
K. G. Stern); alcohols (F. von Falkenhausen and C. Neuberg); aldehydes and
ketones (E. Simon and C. Neuberg); phenols (R. Brieger); organic acids
(J. Schmidt); phosphoric esters (M. Kobel and C. Neuberg); "lipoids"
(A. Winterstein); fats and waxes (H. P. Kaufmann); phosphatides (E. H.
and A. Winterstein); phytostearins (O. Dalmer); penta-, hexa- and hepta-
hydric alcohols (H. Pringsheim and D. Krüger); sugars (H. Pringsheim and
J. Leibowitz); individual sugars (H. Pringsheim and D. Krüger); polysac-
charides (Pringsheim and Krüger).

In addition to methods of identification and estimation each of the articles
dealing with organic substances has an appendix by C. Wehmer, W. Thies
and M. Hadders giving their distribution through the plant kingdom. These
lists seem to have been compiled with considerable care and some attempt
made to separate the dubious from the more satisfactory identifications.

In general the experimental descriptions are clear and sufficient and abun-
dant references are also supplied. It seems a little odd among these to come
upon Strasburger's well-known text-book, cited as an authority for methods
of ash analysis.

It is rather hard to see why a separate section on "inorganic cations and
anions" should be provided as well as that on ash analysis. The two authors
concerned have made little attempt at coordination and a great deal of dupli-
cation has occurred. In view of the very high price, and presumably high cost
of production, severer editing would have been justified. Similarly the sections
on "the physiology of mineral substances" and on ammonia seem out of place:
no sufficient discussion of the very complex data could possibly be given in
the limited space allowed, and the remarks made cannot, indeed, be considered
very critical. These, though, are small points and do not detract from the
great utility to biochemical and physiological botanists and others of the work
undertaken by Professor Dr Klein and his collaborators.

w. o. j.

VON WIESNER. *Die Rohstoffe des Pflanzenreiches.* 4 Auflage. Her-
ausgegeben von PAUL KRAIS und WILHELM VON BREHMER.
Bd I. S. iv + 1122, mit 307 Textabbildungen. RM. 46
geheftet; RM. 49 gebunden; RM. 52 in Halbleder. Bd II. S.
iii + 1131, mit 217 Textabbildungen. RM. 46 geheftet; RM. 49
gebunden; RM. 52 in Halbleder. Engelmann, Leipzig, 1927–8.

"The best dragon's blood comes on the market in the form of tears ('in
lacrimis') the size of hazel nuts." Vol. 1, p. 385.

It is usual in reviews to reserve quotations until the reviewer has defined his
attitude with a reasonable number of introductory remarks. Here, however,
we have a short sentence that so exactly conveys the savour of this delightful
book that pages of earnest introduction could not add a word that would

escape from being superfluous. All the immense store of information, its picturesqueness, even romance, its modernity shot with a slight classical tinge, seem to be summed up in this dozen and a half of words. A better window display could hardly be chosen; if this sample is found attractive the goods within cannot fail to please.

More than fifty years ago von Wiesner first conceived the idea of founding a technology of industrial raw materials of vegetable origin. The result was a "series of somewhat slenderly connected monographs" bound together to form the first edition of this work. His success may be judged from the fact that at the time of his death the author was engaged on a third edition, and that now after only seven years there is a demand for another.

We have the word of competent authority that "to the chemist such a work is invaluable"; also that it "should find a place among the books of reference found on the shelves in all drug stores": it appears, in fact, to have become a standard work among those who buy and sell plant products. Having said so much we may in this place turn to its appeal to botanists, and in so doing we raise inevitably and at once the connection between the pure science and the science in application. A discussion of this matter might easily be endless, and its solution would probably be determined by taste rather than by reason. The taste of a single individual in this connection is, moreover, of no great consequence; but the view, it might even be called the faith, that led to the production of such a book as this and that has dominated its continuance ever since cannot be entirely without interest.

Von Wiesner declared his mind in an introduction that has been but little altered by his posthumous editors. First there is a sturdy declaration of the independence of the daughter science. "The business of merchantile science is to become acquainted with cotton, not with the cotton plant." The starkness of this statement is, however, soon modified by a graceful acknowledgment of the extensive gifts of the mother to her daughter. "With respect to botanical literature"—once more we take the liberty of somewhat free translation—"it is scarcely necessary to labour the point that the technology of plant products is almost wholly dependent on the progress of botany, since it is, indeed, rooted in this science." The rational classification of the materials, as well as methods for their detailed study, derive from the earlier work of botanists; and the older floristic writers, Thunberg, Rumphius, etc., were rich in information of technical value. The most perfunctory turning of our book's pages soon reveals, however, that the debt is mutual. The impetus to several branches of pure botanical study from the applied side has been enormous. Rubber, for example, was little more than a natural curiosity a hundred years ago, but has now become one of the principal articles of commerce. In response to the huge demand the entire plant kingdom has been ransacked for rubber-producing species, latex and its formation intensively studied, and acclimatisation and other experiments undertaken on a large scale. At the present time, it might be added, the industry taxes itself to maintain research that is not tied to immediate commercial values.

In their latest form the two volumes contain twenty-four articles from different pens, each dealing with an important group of substances. A certain degree of uniformity has been achieved throughout, and the majority of the articles pass from a historical survey to a general account of their group, followed by a description of individual materials. A copious bibliography is always appended. Some of the more elaborate sections such as those on fibres (J. Weese and S. Zeisel), woods (W. von Brehmer), and sugars (C. Kallman, W. Krüger, and F. Schneider) reach monographic length. Of these the descriptions and illustrations in the general paragraphs on woods and fibres might well serve as models to many an "Introduction to Plant Anatomy." The article on sugars is a little less fortunate, since in the light of recent work the formulae given for monosaccharides and hence the general account of sugars need revision. A criticism that applies to a good many of the other

sections as well is that their bibliographies are not alphabetically arranged. Lists of hundreds of references in order of their appearance in the text are not handy things; though they are at least better than citations scattered throughout the book at the bottoms of pages, a method that still detracts from the value of many continental books of reference.

To a botanist the various articles are necessarily of very unequal value, those already mentioned being perhaps of the greatest interest. The distribution of emphasis must of course be entirely different from that to which he is accustomed; for example, pigments, many of them degradation products only formed after the death of the plant, properly receive considerable attention. The section devoted to this (by E. Gilg, P. W. Schürhoff, and R. Hofmann) is, however, by no means without interest for the botanist. Accustomed to think of chlorophyll as a rather fugitive substance he will probably learn with surprise that after treatment with copper sulphate to bring it into the more stable "copper chlorophyll" form it even becomes an important article of commerce. Dissolved in vaseline, fats, or oils it is worth up to 15 Rm. per kg., and is used to mask the colour of olive and other oils, especially those used "in Russia for ecclesiastical purposes." It is also used to colour cosmetics, medicines and soaps. In endeavouring to describe the physiological importance of chlorophyll in half a page, the authors seem to have wandered somewhat from the point, besides placing themselves in a hopelessly false position.

Without forgetting the special aim of the book we find it impossible to regard the sections on proteins and enzymes as being of an equal standard with those already quoted. By comparison the treatment is perfunctory in the extreme and could only be justified on the score of a slender commercial importance. The extensive use of enzymes in modern dietetics and medicine as well as in general industry (lacquer, casein, beer, wines, bread, etc.) seem to rule out this possibility at least for them.

It is probably the historical information that will appeal most strongly to the general botanical reader, and a few examples may be acceptable. We learn that the cultivation of *Rubia tinctoria* began in France in the seventeenth century following its introduction by Colbert. During the first republic and empire it was almost allowed to die out, but with the reign of Louis Philippe a great increase began again, the reason being the adoption of red as a general colour for military breeches and the consequent demand for madder. The oldest of the red dyes is probably henna, the true alkanet derived from *Lawsonia alba*. Its use as a cosmetic to colour "the finger and toe nails, the palms of the hands and soles of the feet as well as to redden the hair" has survived from classical times to the modern orient. The Greeks got their best henna from Canopus and Ascalon; the modern soap boilers of Lyons import it from Algiers.

In former times coastal seaweeds were used as food by Japanese, Chinese and the inhabitants of the Atlantic coasts of Europe. In 1920 Sauvageau published the results of an inquiry into the custom in Europe. *Ulva lactuca, Laminaria, Alaria* spp., *Porphyra laciniata, Rhodymenia palmata* and others appear at one time to have been widely used, but the only districts still employing them to any extent in 1920 were the western coasts of Scotland and Ireland. In France, Greenland, Iceland and the Faröes this usage has entirely ceased. On the coasts of Japan, however, thirty species are said to be still employed and a considerable export to China remains in existence.

Enough has probably been said to make clear the impact of these volumes on a botanical mind. In spite of their vast accumulation of up-to-date information it is to the seeker after the curious that they will particularly recommend themselves; to him who desires bright colours with which to bedeck the drabs of description and classification they are a veritable store. The spiritual relationship of their pages to the herbals is greater by far than that of the modern pharmacopœia, and so long as the name of Weisner is served by the piety of editors with reissues such as this it is not likely to fade from botanical memory.

W. O. J.

Plant Sociology: The Study of Plant Communities. Authorised English translation of *Pflanzensoziologie*. By Dr J. BRAUN-BLANQUET. Translated, revised and edited by Profs. G. D. FULLER and HENRY S. CONARD. McGraw-Hill Book Company, 1932. Pp. xviii + 439. With 181 illustrations. Price 27s.

English-speaking ecologists generally will welcome this translation and extension of Dr Braun-Blanquet's *Pflanzensoziologie* which embodies the results of the author's wide experience and extensive knowledge. Such a work is especially valuable as presenting a comprehensive account of the analytical methods of Continental ecologists of whom Prof. Braun-Blanquet is so able an exponent.

It has been said that Ecology is an attitude of mind rather than a special branch of knowledge and if this be true of the subject as a whole it is no less so of its related parts. Whether we focus particular attention on the floristic composition of communities, on the autecology of the constituent species, on their genetical relationships, on the organisation of communities and the life-forms which comprise them, or on the environmental factors and their inter-relations, we cannot obtain any philosophical insight into the problems presented by the sociology of plants without adequate knowledge of all. Each point of view is but a separate abstraction and it is only by their synthesis that ecology can fulfil its major function as a unifying philosophy which more than any other is an integrating influence, not merely between various branches of biology but between these and other branches of human knowledge. We must therefore take exception to "the philosophic foundation," from which the author starts, that "the community has an existence altogether independent of the individual." It may be doubted whether even the converse, that the individual has an existence altogether independent of the community, can be defended on purely philosophical grounds. It is this standpoint which leads to the author's sharp distinction between Sociology and Idiobiology. The present work deals with the former, and though *Plant Sociology* is defined as including "all phenomena which touch upon the life of plants in social units," a sufficiently comprehensive phrase, yet the text displays the author's bias in relegating autecology to a subordinate rôle. Indeed the author states that "the serious study of the problems of synecology is now at its very beginning because the chief interests of most ecologists have centred on autecology. It may, however, be questioned whether the slow advance of synecology is not in fact due rather to our lack of really accurate knowledge as to the autecology of the constituent species of communities, the investigation of which has too often been unduly subordinated to floristic analysis or successional synthesis, so that the prime aim in ecology, namely the causal morphology of plant communities, is lost sight of in mere description.

The subordination of autecology perhaps accounts for the tendency to over-emphasise the implications of recent critical studies on the significance of xerophytic and other morphological features which have been regarded as adaptive. A perhaps not unnatural reaction from the teleological bias of an earlier period. One must recognise that structural and physiological characteristics may be ameliorating factors, even though not a complete compensation, for the disabilities under which the plant exists, whether external or internal, and, indeed, the whole organisation of the individual must be regarded as a compromise between the advantages of specialisation on the one hand and its dangers on the other. It is this which has so often been overlooked in discussions regarding xeromorphic features. Many so-called xerophytes do, it is true, transpire more rapidly than some mesophytes, but it may nevertheless be true also that the xeromorphic features exercise an important restraint. There is experimental evidence to suggest that the transpira-

tion stream is not the almost unmitigated evil that some plant physiologists would have us believe. One may suggest that a certain rate of water loss is, indeed, probably requisite for the adequate transport to growing regions, within a given time, of nutrients which would otherwise only be transported too slowly through diffusion against gravity. If this be true then it is possible that for each species, in a particular set of conditions there may be an optimum rate of transpiration, marked departures from which may have an important bearing on the vigour and distribution of species.

In the earlier chapters the treatment of the characters of vegetation units is rather that of separate abstractions whilst the interrelations between them are scarcely emphasised. This gives a certain sense of artificiality which obscures the three-dimensional picture of the community in the reader's mind. The account of the habitat factors, which occupies about half the book, is a valuable summary of existing knowledge of which the section on the climatic factors is particularly admirable. A fuller treatment of the physical features of the soil would have been advantageous particularly with respect to the water-supplying power of the soil. The statement that the water capacity of soils is more ecologically significant than the water content is misleading since in many localities the water capacity is a potentiality that is seldom realised. Indeed, owing to competition for water between one part of a soil and another, the maximum water capacity, even after heavy rain, may only be maintained for a very short period whilst on the other hand the minimum water content after periods of drought may be far more important ecologically than the mean value, still more than the water capacity.

There are useful chapters on "Soil Types," orographic factors, Biotic factors, and life-forms, whilst the last three chapters deal with the development, distribution, and classification of communities. The classification adopted is based, as to the larger groupings of communities, upon their degree of sociological progression by which is meant the complexity of their organisation. As to the smaller phytosociological units these are characterised floristically. The successional classification of Clements is criticised and discarded on the grounds of its lack of gradation; the physiognomic systems because they depend on appearance. These criticisms, it should be noted, are, however, rather of the method of application than of the principles which these systems attempt to embody. But it must be emphasised that each and all of these systems of classification so far devised, however useful as temporary expedients to meet the needs of our ignorance, are all exclusive in their outlook and fail because, like the discarded systems of taxonomic Botany, they unduly emphasise a single aspect instead of taking cognisance of all. Further, they fail to recognise, what has long since been appreciated in the realm of pure taxonomy, that the value of a character varies with the group to which it is applied; so that neither successional status, nor physiognomy, nor floristic composition, should have equal emphasis throughout the range of plant communities. These communities are the outcome of the interaction of plant and animal life with the edaphic, climatic, and historical factors of the environment, and until communities are studied from all these aspects and each accorded its proper and varying significance our systems of classification are doomed to be of purely ephemeral utility.

But if we have appeared to criticise severely certain aspects of this work it is because we feel that these defects detract from a work which will undoubtedly prove of no small value in the development of the subject.

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RADIAL GROWTH OF THE XYLEM AND THE STARCH RESERVES OF *PINUS SYLVESTRIS*: A PRELIMINARY SURVEY

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(With Plates II and III, and 1 figure in the text)

Introduction

A RECENT review of the literature (9) has stressed the significance of data relating to the seasonal inception and maintenance of cambial activity in different regions of the tree. In the case of the soft wood or Conifer, such data are very deficient, and it is difficult to know what weight to attach to many recorded observations which are based upon occasional samples from a few localised regions upon the tree. During 1927–30, therefore, extensive observations were made upon the cambial activity of *Pinus sylvestris* L., upon allied species and upon other Coniferous genera. As a result the following notes upon the initiation and cessation of radial growth of the xylem and upon changes of the starch content in the axis of *P. sylvestris* L. are placed upon record.

The results are based primarily upon the more intensive observations on this species during a period of twelve months, but it is possible to discuss these observations briefly in the light of a much wider series of observations which were carried out upon other species and genera during the three-year period over which this study extended.

Material

The observations described in this paper were made on vigorously growing 20–25 ft. pines, approximately 20 years old. These trees were growing in an open uniform stand, 5 miles from the centre of Leeds, on peat, with a hard layer of "pan" 2 ft. below the surface.

One tree was felled about every 4 weeks. The following table gives the times of felling. During 1928 no tree was felled between April and September, and to make good this deficiency a tree was felled in May, 1929. Owing to the fairly wide intervals between the felling of the trees, any difference in this individual, due to seasonal differences, should be nullified.

Tree No. 1. Felled on January 22nd, 1929.
    ,, 2.   ,,   February 29th, 1928.
    ,, 3.   ,,   March 19th, 1928.
    ,, 4.   ,,   April 12th, 1928.
    ,, 5.   ,,   May 6th, 1929.
    ,, 6.   ,,   September 11th, 1928.
    ,, 7.   ,,   October 15th, 1928.
    ,, 8.   ,,   November 29th, 1928.
    ,, 9.   ,,   December 25th, 1928. This was a large mature pine growing in an open stand, 20 miles north of Leeds.
    ,, 10. Examined at frequent intervals.

Tree No. 10 was one of a stand of 40 ft. pines (50–60 years) growing in the same locality as the smaller trees. Tree No. 10 was first examined on May 6th, 1929, and then at close intervals during the opening and closing phases of growth.

A bi-weekly series of uniform nursery trees was studied over the period May 14th, 1929 to July 25th, 1929. These trees were 2 year–1 year *P. sylvestris* and were obtained from the Forestry Commission nursery at Pickering, by courtesy of H.M. Forestry Commissioners. In addition to this routine material, samples were taken from the branches, trunk and roots of numerous trees of *P. sylvestris* growing over an area of approximately 200 square miles, during the period 1927–9, and in no case has any exception to be placed on record to the observations as presented in this paper.

METHODS

From each of the felled trees samples ($1\frac{1}{2} \times \frac{3}{4}$ in.) including 1–6 wood rings, were taken at intervals of from 6 in. to 4 ft. along representative branches from each whorl, down the trunk and along at least three roots from the root system—in all cases the whole root system was excavated, and in the majority of cases the roots were followed out to their growing apices. Between the felling of the different 20 ft. pines a constant check was kept on the progress of cambial activity by examining branches, surface roots, and small samples removed by means of a sharp knife from the trunks of

other trees in the vicinity. Tree No. 10 was left *in situ,* and small samples, about $1 \times \frac{1}{2}$ in. and including 1–2 wood rings, were removed at definite points along the branches and down the trunk. From time to time a single vigorously growing surface root was removed from the tree. The nursery trees were preserved entire. Every sample was kept during the whole period of the investigation and the position of each sample on the tree was recorded in an index. Sections were cut, without previous imbedding, on a Reichert sliding microtome. About 3000 permanent preparations and about three times this number of temporary mounts were made and examined. Branch and root samples were always taken from the upper surfaces. Trunk samples were all from the same side of the trunk, control samples being taken also on the north, south, east, and west faces. No difference with regard to *time* of initiation or cessation was noted in these control samples, neither did the judicious removal of small samples from tree No. 10 make any appreciable difference to the normal progress of growth.

Criteria

It is usual in work of this nature to base conclusions on data of the following type:

> (1) Number of rows of new tracheids formed.
> (2) Radial diameter of the new zone of wood.
> (3) Type of tracheids formed.

These are criteria of limited value, for ring width varies greatly at different points on the trunk (MacDougal(8)) or on a branch. It is evident that, other things being equal, there will be a greater intensity of growth in the region of the widest rings, and therefore tracheid counts or measurements are not suitable criteria for comparing one region with another when determining inception or cessation of cambial activity. Further, the width of the cambium, the number of cambial cells and their dimensions in transverse section vary with ring width (Brown(1), p. 203).

Much of the literature relating to cambial activity is burdened with data such as are indicated above, which unfortunately are of little value and have resulted in very varied opinions. A comparison with previous years' rings is sometimes suggested as a means of overcoming local differences, but the chief objection is that the ring width rarely remains constant. The crux of the matter lies in securing and accurately identifying the first signs of initiation and the last signs of cessation of growth. This necessitates the use of criteria

based on the state of the cambial layers themselves, and such criteria alone have been used to determine the inception and cessation of xylem growth during the present investigation.

Complete rest is denoted by thick radial walls and tangential walls of a uniform thickness. The cambial cells have an almost geometrical outline and the layer looks exceedingly solid (Pl. II, fig. 1). With active division this regularity is lost; thick radial and thin tangential walls are present. Eventually, the wide newly differentiated tracheids merge insensibly into the zone of narrower cambial elements. This latter becomes very wide, in extreme cases from 30–40 cells deep (Pl. II, fig. 4 and Pl. III, fig. 5). When activity first begins the inner row of cambial cells swells up and differentiates without division. The other cambial cells begin to swell and lose their regular arrangement. These cells are not all of a uniform width and new tangential walls are visible.

Pl. II, figs. 2 and 3, illustrate the beginning of cambial activity. Whilst the cells immediately outside the xylem are simply increasing in size, in fig. 2 new and thinner walls are visible in the cells just outside these. In fig. 3 several rows of differentiating cells, just outside the xylem, have ceased to divide, but at the top of the photograph cells are visible containing new, very thin, tangential walls.

If these photographs are carefully compared with Pl. III, fig. 6, which is taken just as cambial activity is ceasing, certain differences will be apparent. In Pl. II, fig. 2, the layer of differentiating cells, on the face of the lignified wood, are more expanded radially.

The maximum contrast which held (during the winter dormant period) between the last completely differentiated cell and the cambial cells along the same radial file, is now replaced by a progressively wider gradation in size and character between the last wood cell to be completely differentiated and the smallest cambial cell along the same radial series. It is thus difficult to distinguish exactly between cambium and wood or to identify a particular peripheral ring of tissue as the cambium. This is apparent from Pl. II, fig. 3, and this appearance is never obtained towards the close of cambial activity. At this time the differentiation of cells already produced begins to overtake the formation of further cells, so that maximum gradation progressively gives way to maximum contrast. A peripheral ring or zone of two or three rows of narrower, uniform and definitely thinner walled cambial cells, extending across all the radial files, thus becomes visible, before division has entirely ceased and before actual lignification begins in what will be the last wood cells of the season. The

result is to give the cambial region in Pl. III, fig. 6, a somewhat more regular appearance than in Pl. II, figs. 2 and 3.

In the very last stages lignification is slow and the outer tangential wall lignifies last. Once this is lignified growth is completely over. Differentiation of the xylem may slow down, however, till only the outer walls of the last elements are unlignified; growth may then start again. Such a stage may be distinguised from initiation after complete rest, by the fact that lignification at once commences in the freshly formed tracheids. The initiation of growth after a marked period of rest always means that more than one row of tracheids, usually three or four, are fully differentiated before lignification begins.

Changes in the cambium are usually compared with external changes of the buds, i.e. bud swelling and bud break. These latter are rough and ready conceptions at the best, for many important changes of small magnitude may be taking place in the bud long before there is any swelling visible. Examples may be found of vigorous xylem differentiation in many or all parts of the woody axis of a tree without any signs of bud swelling, as for example in *Abies grandis* (Californian Red Fir) when grown in Yorkshire, yet changes comparable with those occurring in the bursting or open buds of other species may easily be taking place in these apparently "dormant" buds. A point of interest is that the buds of *P. sylvestris* are not homologous with the buds of many other Conifers or with those of hardwood trees. By November the terminal buds of the following season, equipped with scarious scales, are formed within the obvious buds of *P. sylvestris* (cf. Ward(12)). Detailed study is therefore necessary before comparisons can be instituted between the relation of growth changes to bud swelling and bud break in pines and other trees.

RESULTS OF THE INVESTIGATION

(1) *Xylem growth in the trunk and branches*

The beginnings of growth were examined in greatest detail in tree No. 10. On May 6th the preliminary swelling of the cambium was evident all down the trunk, though there were no signs of the buds breaking. Between 10 and 14 ft. from the top of the tree two rows of tracheids were differentiated and the cambium was showing more signs of division than elsewhere. At the base of the trunk the amount of growth was rather less than elsewhere. One row of tracheids was being differentiated, but the radius of these tracheids

was not so great as in other parts of the trunk. This basal part of the trunk was the region of the narrowest rings, whilst that part of the trunk between 10 and 14 ft. from the top of the tree had wider rings than elsewhere.

By May 24th the buds had burst and about $\frac{1}{2}$ in. of new growth was visible. There was now a definite gradient of xylem growth down the trunk and ten successive samples taken from 6 ft. below the apex to the ground level showed the following numbers of fully differentiated tracheids:

<div align="center">

4–5

4–5

3–4

1–2

1–2

1

1

1

1

1

</div>

The first signs of lignification were just visible in the angles of the tracheids in the upper part of the tree. These first signs of lignification were in a falling gradient down the trunk, the amount of lignification dropping off rapidly below the branches. According to Brown there is also a similar gradient of summer wood formation in *P. rigida* (2), and the author has extended this observation to *P. laricio*. The local differences observed on May 6th had been smoothed out in the production of the gradient noted on May 24th. Tree No. 4, felled in April, showed an earlier start, and the samples secured showed more radial growth than did those taken from tree No. 10 on May 6th. There was every indication, however, that the first signs of growth in the trunk had occurred simultaneously from below the terminal bud to ground-level. In this series of trees marked growth continued until July. In September growth was still taking place all down the trunk, both in tree No. 6 and in tree No. 10, and summer wood formation was now general. In October tree No. 7 was still growing along the whole length of the trunk, though growth was evidently very slow in the apical regions. Growth had only just ceased in tree No. 8 by November 29th. There were abundant indications that cambial activity had ceased very recently in this tree, probably from 7 to 14 days before it was felled. In all probability growth had ceased at the same time down the whole length of the trunk.

In describing growth in the branches it is simplest to begin with the buds. Towards the close of a growing season xylem growth is very sluggish immediately below and within the buds, but it appears to continue until unfavourable conditions bring it to a close. Throughout the winter partially lignified tracheids may be seen in the bud, and consequently whenever conditions become at all favourable, some little further differentiation is to be expected. With the resumption of growth in the trunk there is none at all in the major part of the branches (tree No. 10) but change is occurring *within* and at the *immediate base* of the closed bud, which at this time shows little or no elongation. Such change does not extend at the most for more than an inch or so below the bud and only consists in the preliminary swelling of the cambium. Hence it appears at first sight that the inception of growth in the trunk of a mature tree, such as No. 10, which has no leader, is not directly connected with growth changes at the bases of the branches. However, on May 6th the bases of only two (major) branches of tree No. 10 were examined and therefore growth may have reached the base of some small branch in the much divided upper parts of the tree. All the buds examined show that similar changes occur within the buds to a more or less marked extent at about the same time. In a tree with a leader, similar changes take place simultaneously within the terminal bud and coincide with the first signs of swelling all down the trunk.

In an individual branch, growth begins within the terminal bud and goes slowly down the main axis of the branch (the secondary branches have not been examined) towards its base. This downward rate of transmission varies in different branches of the same tree (i.e. it depends on the age of the branch). A definite down gradient of growth was observed along the branches of trees Nos. 5 and 10. It was definitely established from these that the preliminary swelling of the cambium progresses slowly along the branch from *within the bud* and that lignification of the tracheids so formed begins *within the bud* but nowhere else, and may commence there before the swelling of the cambial cells has reached the base of the branch.

By deduction from the state of growth in tree No. 5 (on May 5th) and of tree No. 10 (about a week later) it was decided that pronounced growth first became visible in the large branches of both young and mature trees (apart from the immediate vicinity of the buds) about 2 weeks later than growth in the trunk.

In tree No. 5 the apical whorl of branches showed radial growth along the length of the branches. At the apex, below the bud, one

row of unlignified tracheids was cut off. At the base, two rows of lignified and three of unlignified tracheids were observed. Branches in the middle region of the tree showed a swelling of the cambium along their whole length, whilst in the lowermost whorl the cambium was completely dormant towards the base of the branch.

Vigorous growth was evident in September in all the branches of tree No. 6. This, however, was somewhat of an anomalous tree. Its growth was unusually vigorous and prolonged, and there were indications that a second burst of activity had occurred. Tree No. 7, felled on October 15th, showed cessation stages in all its branches, and it therefore appears probable that branch growth begins to fall off during September. The branches up to 12 ft. above the ground level had completely ceased growth. Branches at $14\frac{1}{2}$ ft. above ground level showed the very last stages of growth uniformly along their length. At 18 ft. above the ground the branches were showing the last stages of lignification, also uniformly along their length.

By November 29th all the branches of tree No. 8, as well as the trunk, had completely ceased growth. No further indications of growth were evident until the following spring.

Although the 20 ft. pines showed great regularity in the order of inception and cessation of growth in their branches, the mature pine (tree No. 10) showed irregularities which may be connected in some way with the branch systems. All the young trees had regular whorled systems of branches, whilst in the mature pine (tree No. 10) the branches were confined to an irregular asymmetric head without a leader.

(2) *Xylem growth in the roots*

Particular attention was paid to the roots. In the spring complete root systems up to the growing apices were unearthed at weekly, or, at the most, fortnightly, intervals. On each occasion one root was taken from tree No. 10 and other roots from several comparable trees in the same stand. The root system of a mature tree shows a thickened basal part adjacent to the trunk and about 2 ft. long. This part suddenly thins off to the root system proper and, so far as radial growth is concerned, the thickened part may almost be considered as part of the trunk.

The first really discernible sign of swelling of the cambium in the root occurs about a fortnight later than it does in the trunk (excepting the thickened basal part described above, which shows swelling of the cambium about a week later). The swelling is almost imperceptible and is barely discernible. It appears more marked in

the proximal regions, but in no case has it been found in the thicker parts of the root without corresponding changes on a smaller scale occurring in the thin parts of the root. Swelling is very slow and by May 24th it had not reached a stage even comparable with that obtaining in the earliest stages of trunk growth. Up to the beginning of June the further changes were slight and insignificant. Roots examined on June 13th showed a sudden spurt of activity and for the first time showed growth changes comparable with those incident on growth initiation in the trunk. On this date two roots from tree No. 10 and two from another tree showed uniform growth. Those from tree No. 10 showed uniform activity up to the growing apices. The cambium was swelling and the first row of tracheids was just about to be cut off. In the roots from a separate tree there was vigorous growth in the root-trunk base, 20–30 rows of new tracheids being present. One foot further along the root only two rows were present. This was in the thickened proximal region of the root proper. Between points 4 ft. from the base of the trunk and the root apices 12 ft. away, growth was uniform and one row of tracheids had been differentiated. Thus, excluding the region common to trunk and root, and perhaps the proximal foot or so of the root proper, the beginning of growth occurs evenly along the root. The first signs of lignification were only seen in one root, and there lignification became evident in the proximal parts and close behind the growing apex before it did in the middle regions.

By the beginning of September growth of the thin distal parts of the roots of tree No. 10 and of tree No. 13 was entirely over. It appeared to have ceased about the end of August, or probably earlier in some roots. Young seedlings examined at intervals of 3–4 days provided details of the root-growing period for nursery trees. The first tree was examined on May 7th. Root growth had just started and the aerial parts had apparently been growing about a fortnight. Growth in the distal parts of the roots of these trees ceased about July 10th; the roots thus have a growing period of about 10 weeks, from the beginning of May to the end of July. As in all other respects young pines have a longer growing period than old ones, it appears that radial growth in the thin parts of the roots of a mature tree is over at the latest by the end of August—probably earlier—and that at the most they have the same growing period as those of young trees, namely about 10 weeks. Different parts of the root system probably behave differently, and some parts may cease quite soon, e.g. surface roots of *P. laricio* were observed to cease growth in their

distal parts by June 22nd. Growth, however, persists for a longer period in the thicker parts of the root. For example, in tree No. 7 in October, at a point 10 ft. from the base of the trunk, there was no growth in the finer roots. At 5 ft. from the base, growth was just ceasing and the last row of tracheids was partially lignified but still with protoplasmic contents. Within 3 ft. from the base vigorous growth was evident, which increased towards the more proximal regions where it was about equal to that in the stem. Roots examined in November from tree No. 8 showed a complete cessation of growth, as also did roots collected from another tree on the same day. From November to May the whole of the root system is completely dormant with respect to radial growth of the xylem.

The tree felled in September (No. 6) which was showing an unusual vigour of growth, provided abundant proof that the cessation of radial growth in roots takes place in a basipetal sequence. In a surface root growth had practically ceased 5 ft. from the base of the trunk. At 4 ft. from the base there was vigorous growth with but three rows of summer tracheids. At the base of the root the cambial zone was very wide with active cell division. All the other roots of this tree showed a similar mode of cessation. Many of these roots were followed out for distances of nearly 20 ft., but not the slightest indication of growth in their distal parts was forthcoming. Roots from tree No. 10 in September and from other comparable trees growing as far as 20 miles away, all showed most definitely that the sequence of cessation in the root is from the growing apices towards the base of the trunk. All the young seedling roots showed a similar mode of cessation, and in mature trees the phenomenon was observed over two successive seasons. Growth is over comparatively rapidly in the young roots, but persists longer in the thicker and more proximal parts; moreover, it apparently persists for longer periods the thicker the root grows, year by year. The phenomenon is thus cumulative.

The roots of *P. sylvestris* show two periods of growth in length. The first is early in the season, May–June, and the latter at the end of August and the beginning of September. In the former period the production of fibrous roots seems to predominate, though there is also some growth in length of the stout root leaders. These grow in length most vigorously during the later period of root growth. With the inception of radial growth in June, growing apices of the roots were obtained, but there was no indication of any acceleration of radial growth in their vicinity. With the second burst of length

growth in September a careful examination was made of the growing apices. These latter were found to have no effect on radial growth beyond the one-year wood. Determinations are difficult to make in this region, as the rings of wood are not complete due to the formation of resin canals, but most certainly not the slightest effect on radial growth is visible at any greater distance than 2 in. behind the new growth.

Summary of the growth changes

A complete picture of radial growth in *P. sylvestris* shows it starting up suddenly and uniformly in the trunk towards the end of April and the beginning of May. At the same time, radial growth is resumed in the buds. This is about a week before the latter show any appreciable lengthening. Meanwhile radial growth is gradually becoming evident further and further away from the bud. With the bursting of the buds a gradient of growth and lignification appears down the trunk. By this time, growth has reached the bases of the upper branches and lignification has become apparent down the whole length of some of these branches. In young trees growth becomes apparent, first in the upper branches and last in the lower ones. This vertical and downward sequence is apparent and not real, for growth began in all the terminal buds at once. The apparent order is due to the much slower rate at which growth changes progress down the lower branches, together with their greater length. Changes in the root proper are scarcely discernible until some 6 weeks after growth has started in the trunk. When marked growth does start it is very sudden and is simultaneous along the whole root. In the thinner parts growth is vigorous (Pl. III, figs. 7, 8) and is over rapidly (10 weeks). In the thicker parts growth ceases more slowly and the cessation progresses towards the base of the trunk, so that growth in the regions common to root and trunk ceases at about the same time as it does in the aerial parts. The slowing down of growth in the aerial parts is more irregular. In the trunk the vigour of growth seems to fall off earliest in the upper parts. A complete cessation of growth occurs everywhere at about the end of October. The tree thus has a growing period of about 6 months.

(3) *The starch reserves*

Material and methods

The material used for the study of cambial activity was also used for investigating the starch reserves. Sections of a standard thickness (100 μ) were cut. These were washed for 5 min. in water to

remove the excess of spirit, stained in an excess of a standard concentrated solution of iodine in an aqueous solution of potassium iodide, and mounted in a mixture consisting of aqueous iodine one part, pure glycerine two parts. To facilitate easy interpretation of the results, numerical values were assigned to the different degrees of blackening produced by the iodine-starch reaction (as seen under the microscope). The criterion was mainly one of colour reaction, but the size and distribution of the grains within the cells of any particular tissue was also taken into consideration. From the figures so obtained the qualitative fluctuations in starch were plotted (Text-fig. I). In
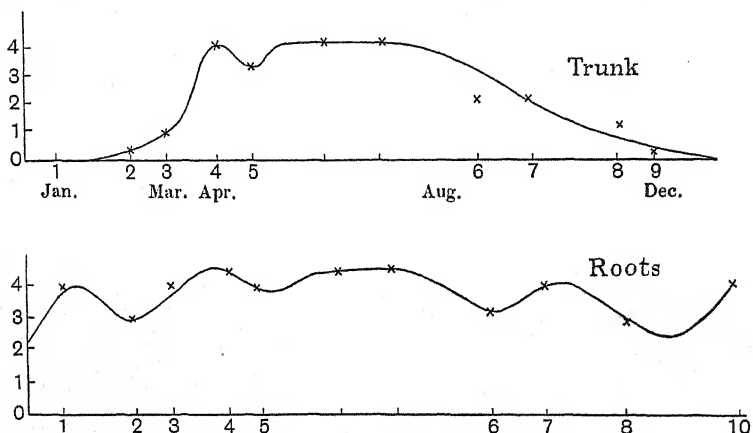
Text-fig. I. Curves of seasonal starch distribution in *P. sylvestris*. The ordinates are based on an empirical qualitative scale as described in text.

order to prevent any personal bias creeping in, the material was only studied when a complete collection had been made, and it was then examined in a haphazard order. The numerical values of the observations were entered in tables and the nature of the fluctuations did not become apparent until the curves were subsequently constructed. The accompanying curves represent the average of many. At the close of the investigation 39 different curves were constructed for different individuals and for different parts of these individuals. The "estimations" refer to the old phloem (excluding that of the current season only) and to the cortical tissues up to the phellogen, as these tissues contain the major part of the reserve starch.

Results

The general results will be seen best from the curves (Text-fig. I). That for the trunk represents the middle part of the trunk, but may be taken as applying to the whole of the main axis and giving the

general nature of all major fluctuations in the aerial parts. That for the roots is typical of their thin and distal parts: towards the thicker proximal parts the curve more nearly approaches that for the aerial parts. In January it will be seen that in the trunk the starch content is nil. There is a slow accumulation of starch from February until the end of March, after which there is a sudden rise to a maximum at the beginning of April both in the root and the shoot. This maximum is maintained until the end of July, with a slight but transient minimum in the trunk about the beginning of May. From the end of July or the beginning of August the starch content slowly drops off to a winter minimum which begins early in January and lasts till the end of February. It will thus be seen that the period of maximum starch reserves coincides with the period of most vigorous radial growth. Starch suddenly appears just about the time that cambial activity sets in and is present in large quantities throughout the summer. At the time cambial activity slows down the starch reserves begin to disappear.

The winter minimum is very pronounced: starch is totally absent from all the stem tissues, but the roots show only a comparatively slight drop. *Starch is never totally absent from the roots*, and during the summer maximum these latter show a higher starch content than the trunk. There is generally a marked rise in starch towards the growing apices of the roots.

The slight and transient minimum which occurs in the stem during the general summer maximum is very interesting. It is apparent that this slight drop must have taken place fairly rapidly, though over what period the change occurred it is difficult to say, owing to the considerable intervals elapsing between the examination of the different trees. Also, the particular tree showing this drop is inter- polated in the series from another season and the objection might be raised that it is not truly comparable. A comparison with the fluctuations in tree No. 10 over this period is therefore of interest. Samples were taken from this tree at the beginning and the middle of May and at the beginning of June, and examined for starch. The general changes were about 2 weeks later than those occurring in the younger trees. On May 6th the whole tree was filled with starch and the summer maximum had obviously set in. About a fortnight later, on May 24th, a slight drop was noted in the trunk and towards the apex of the roots. Three weeks after this the whole trunk was again filled with its maximum quantity of starch. Thus, it would appear that during the summer there is a sudden but slight drop followed

again by a rapid reappearance of the starch. The data from tree No. 10 indicates that the fall in starch content to a condition comparable with that in tree No. 5 takes at the most 2 weeks and that it rises to its normal summer maximum again in 3 weeks at the most. The study of the 3-year nursery trees threw some light on these changes. In these trees the summer maximum had been attained by May 7th and about 4 weeks after this there was a slight drop throughout the tree. This slight depletion of the starch took place over a period of 14 days. The loss was made good and the starch back to its original maximum value 7 days later. Objections may be raised to the application of these results to larger trees, but, bearing in mind the distinct correlation between the fluctuations going on in the small trees and those proceeding both in the 20 ft. pines and in a 40 ft. individual, it seems permissible to make a complete story from the whole of the material studied. In general, then, there is a total absence of starch from the trunk during January and February. In the early part of March the trunk is slowly filling with starch. Towards the end of March the tissues of the trunk suddenly fill with starch and by the beginning of April the summer maximum is attained. About May–June (depending on the tree) there is a slight and very sudden drop in the starch reserves, which rapidly rise again to their maximum. This latter (the "summer maximum") is maintained until the end of July or the beginning of August, when starch slowly begins to disappear. The fact that the maxima and minima do not coincide in time exactly in the different material studied is not of great consequence. The seedling trees began growth much earlier and had a longer growing season, yet both the 20 ft. pines and the seedlings agreed in showing a small summer minimum *about 4 weeks after the starch maximum had been reached*. Unfortunately, material was not collected early enough from tree No. 10, but from a comparison with the younger trees it would appear that the small drop in starch content also occurred about 4 weeks after the sudden reappearance of the starch. Thus the curve for the trunk, whilst conforming to the observed values for the 20-year pines, has been modified in the region of "summer minimum" in the light of the above knowledge.

In addition to trunk and roots, the starch content of branches and leaves was studied in detail, but for present purposes it may suffice to summarise the results of these observations as follows.

In the trunk, branches and roots there is a winter minimum and a summer maximum of starch. During the summer maximum there

is a slight fall of short duration. Starch is stored in the old leaves and fluctuates in quantity along with that in the branches. The young leaves within the bud contain starch, but this soon disappears. Small quantities are again accumulated, but these disappear with the slight fall during the summer maximum. After this the young leaves rapidly fill up with starch. Two-year-old leaves contain more starch than one-year-old, three-year-old more than two-year-old leaves. The roots have a periodicity of their own, on which the stem fluctuations are apparently superimposed.

DISCUSSION

The results are to be regarded as a preliminary survey, as the entire cycle of growth was studied in detail over one season only. Conclusions are based strictly on the observations, though the latter might with advantage have been made at more frequent intervals. The conclusion that radial growth is initiated simultaneously along the trunk, and later and simultaneously along the roots, is, in the author's opinion, justified on the available data. These data do not preclude the possibility of an exceedingly rapid "wave of initiation" of radial growth proceeding down the trunk, and from the base of the trunk along the roots, the effect becoming less apparent as the "wave" progresses towards more distal regions. In contradistinction to this idea, and to certain of the data already presented, are Brown's observations. Brown noted a separate inception of growth in the trunk and branches of *P. rigida* [2]. In each the point of inception was some distance behind the apex, and growth was said to progress in both cases in a basifugal *and* a basipetal direction from the point of inception. This is definitely not the case for *P. sylvestris*, though local differences in the *amount* of growth are apparent exceedingly early. A rapid resumption of growth all over the trunk and a slow basipetal initiation down the branches is in accord with Brown's published data. According to Brown ([2], p. 394) growth is resumed all over the main axis of *P. rigida* in 19 days, and the conclusions presented by Brown for *P. Strobus* [1] are by no means in opposition to a uniform cessation of growth. Root growth is stated by Brown to begin much later than trunk growth, and his data ([1], p. 236) show that noticeable radial growth of the roots begins at least 4 weeks later in the roots than in the stem.

With regard to the observations on the relation between radial growth and the phenomenon of "bud break" (p. 81), the observation of Brown [1] that the radial growth of *P. Strobus* precedes

elongation growth is of great interest. This certainly appears to be the case if one considers only the obvious buds of *P. sylvestris*.

Regarding the second object of this paper, the only justifiable conclusion is that there is no relation between the major movements of starch reserves and the progress of cambial activity in *P. sylvestris*. Correlations between starch reserves and cambial activity have been attempted previously for the hardwoods. A typical fluctuation of starch in the hardwoods was worked out by Cockerham (4) for *Acer* and similar results were obtained by Fischer (5), by Cameron (3) for the pear and apricot, and by Swarbrick (11) for apple. Cockerham found that starch was at a minimum from October to February, rising to a maximum in March. Unlike *Pinus* this maximum in *Acer* falls off rapidly, and by the beginning of June the starch content is at a minimum and rises again to a second maximum in August. The small and transient summer minimum, barely discernible in *Pinus*, must represent this considerable drop in the hardwoods. Swarbrick (11) thought that there was a close connection between starch disappearance and cambial activity. No such correlations as those put forward by Swarbrick can hold for *P. sylvestris*, for in the latter starch is present all down the trunk and along the branches during the period of greatest radial growth, and the starch does not sensibly diminish in amount till the intensity of radial growth is slackening off.

An examination of the literature shows that all our existing information as to starch movements in relation to cambial activity in the Conifers has been obtained through defoliation experiments. For example, Hartig (6) defoliated a Weymouth pine, and found that under these conditions radial growth caused a depletion of reserve starch. Although it took two seasons for this latter to disappear completely, the radial growth during the first season of defoliation was considerably less than normal. Again, Jost (7) found that removing the elongating buds from *P. laricio*, which shows comparable starch fluctuations to *P. sylvestris*, caused radial growth to be reduced by one-half, although the food reserves must have been adequate for the formation of a much wider ring. Jost does not mention the effect of this treatment on food reserves, although the radial growth no doubt took place at the expense of reserve starch in the absence of the young leaves. Jost found that the removal of the old leaves, with the buds left *in situ* did not reduce, but rather increased, radial growth, although the old leaves contain large quantities of starch. Defoliated pines showed normal radial growth if the terminal bud was left on.

To sum up the available evidence. That presented for *P. sylvestris* precludes any generalisation that cessation of cambial activity is due to the accumulation of starch in the woody parts. Depletion of starch is perhaps proportional to radial growth in cases of defoliation, but this cannot be said to be a general phenomenon of cambial activity under normal conditions. The presence of starch is not causally connected with cambial activity either in one way or another. Buds and young leaves are evidently of importance, indicating, especially in view of defoliated and normal trees, that a food supply from young leaves may be necessary for radial growth. The two processes, starch movement and cambial activity, show nothing of the nature of a causal relationship, but rather appear to be two distinct seasonal cycles—a growth cycle of activity and dormancy and a physiological adjustment of the starch-sugar equilibrium. Length growth, as Schellenberg (10) shows, proceeds at first at the expense of reserve starch, thus accounting for the observed sub-apical depletion in *P. sylvestris*, but this depletion does not affect the seasonal starch fluctuation—it is rather imposed upon it.

It is difficult to dissociate the defoliation experiments of Hartig and Jost from some unexplained influence which the presence of buds appears to exert on cambial activity. There are, however, several interrelated phenomena in the growth of *P. sylvestris*. There is, independent of all other considerations, the connection of radial growth with growing buds, merely as such. This connection of growing organs with cambial activity appears to be very intimate in many hardwoods, though even there dubious cases occur. In the Conifers the connection does not appear to be so intimate and activity may start in the absence of visible bud break. There are the experiments of Jost which indicate a connection between leaves and radial growth, which connection is more readily appreciated than the seemingly abstract influence of the opening bud. Finally, in the absence of leaves, radial growth is still possible, but it then takes place at the expense of the reserve starch and is often less than normal.

Summary

The aim of the investigation was to determine the order of initiation and cessation of radial xylem growth in *P. sylvestris*. Both aerial and underground parts were considered. The seasonal changes in starch content were examined and the relation of these changes to radial growth considered.

Growth in the trunk begins suddenly, and, as far as could be determined, uniformly along its length, at about the beginning of May and before the buds burst. The most vigorous growth in the trunk occurs from May to early July. After this, slow but continuous growth continues till the beginning of October. Root growth starts some weeks later than trunk growth.

The impulse to growth begins uniformly along the length of the root. Growth in the thinner distal parts of the roots is over comparatively early (growth lasts 2 months at the most) and growth ceases first at the root apices. The order of cessation is from the root apices backwards. Growth in the proximal parts of the roots continues almost to the end of the trunk-growing season. At the same time a "wave" of cessation of growth gradually progresses towards the proximal part of each root.

The study of the buds was confined to the "one-year" shoot within the bud. Buds all over the tree begin growth at the same time. In each branch the initiation of growth is within the terminal bud. The initiation of growth within the bud is coincident with the first signs of growth in the trunk, both in trees with a "leader" and in mature pines without a leader.

Both swelling of the cambial cells, and cell division, spread down the branches from within the buds. This basipetal progress of activity is rapid in the young branches and slower in the older branches; it is very slow in the lowest and oldest branches, so that unless the apices of the branches are examined at the beginning of the growing season, the uppermost branches on the tree *appear* to begin growth first. When radial growth reaches the bases of the uppermost branches, an impulse to growth is sent down the trunk.

Lignification of the new wood begins in the buds, and lignification progresses towards the proximal parts of the branches. In the trunk, lignification begins at the apex and progresses towards the roots.

The oldest branches cease growth first; in each branch the final cessation of growth takes place in all parts of the branch at about the same time.

The proximal parts of the roots, the whole of the trunk, and the uppermost branches, all reach the final stages of growth at about the same time.

The starch reserves in the aerial parts show a winter minimum, with total absence in January. Starch is at a maximum from April to the end of July, with a slight fall of short duration in May. This slight drop represents the considerable depletion already recorded by

Cockerham for *Acer*. From July to the end of December starch is slowly disappearing. Starch disappearance and reappearance begins first in the leaves and branches.

In trunk and branches both starch appearance and starch disappearance seem to be uniform along their length.

The roots show starch fluctuations of their own, on which the stem fluctuations are apparently imposed.

Starch reserves flood the young leaves in the early part of July. After this, starch in the leaves fluctuates in sympathy with the rest of the tree.

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EXPLANATION OF PLATES

PLATE II

Fig. 1. Resting stage of cambium of *P. sylvestris* shoot. The cambial cells lie between *C–C'*, forming a zone of apparently similar cells. These cells divide but there is no division in the cells below *C*.

Fig. 2. Early cambial activity in shoot of *P. sylvestris*. *C–C'* are cambial cells. Below *C* is a layer of young cells in which no division occurs. The cells merely increase in size and differentiate into xylem elements. (The first differentiation of phloem elements occurred previous to xylem differentiation and again began without cell division.)

Fig. 3. Early cambial activity in shoot of *P. sylvestris*. *C–C'*, the layer of actively dividing cambial cells, is further away from the old wood. Next to the xylem are several rows of expanding, differentiating cells which have ceased to divide.

Fig. 4. Active cambium in shoot of *P. sylvestris*. Cell division and differentiation are equally active, and the cambial cells merge gradually into young xylem and phloem elements. Hence the cambial layer can no longer be clearly delimited.

PLATE III

Fig. 5. Enlarged photograph of Pl. II, fig. 4.

Fig. 6. Cessation of cambial activity in *P. sylvestris* shoot. Differentiation of new xylem proceeds more rapidly than division of cambial cells. Hence a distinct zone of thin-walled cambial cells is marked off from the wide, fairly well-differentiated cells last cut off.

Fig. 7. Resting stage of cambium in root of *P. sylvestris*. In the dormant cambium of small, fine roots, the zone of apparently similar cells (*C–C'*) is often a uniseriate layer.

Fig. 8. Vigorous activity in root of *P. sylvestris*. Wide zone of actively dividing and differentiating cells.
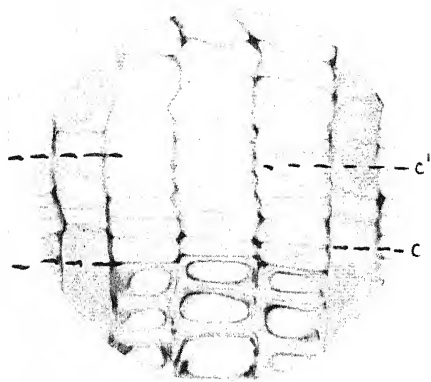
Fig. 1
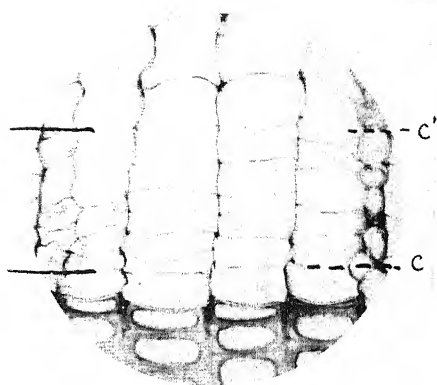
Fig. 2
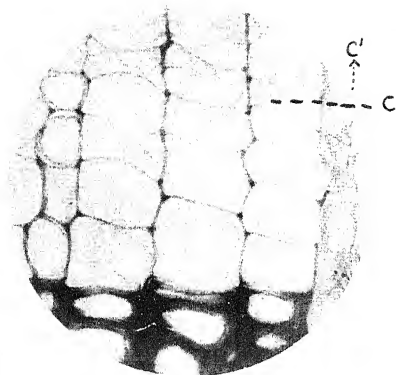
Fig. 3
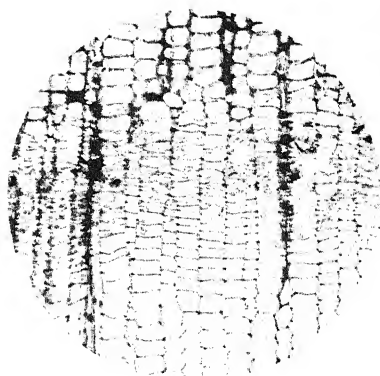
Fig. 4

WIGHT—RADIAL GROWTH OF XYLEM, AND STARCH RESERVES OF
PINUS SYLVESTRIS
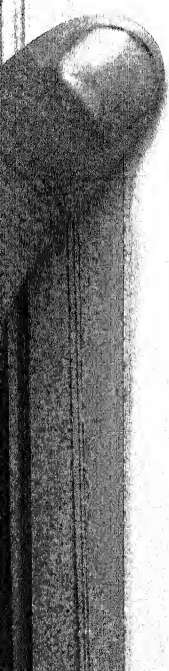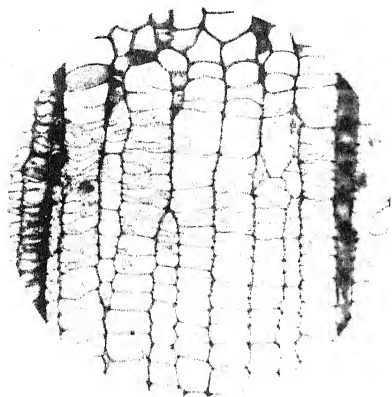
Fig. 5

Fig. 6
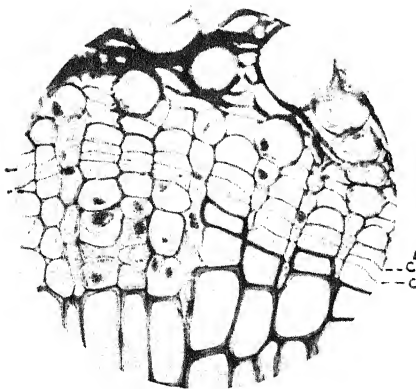
Fig. 7

Fig. 8

WIGHT—RADIAL GROWTH OF XYLEM, AND STARCH RESERVES OF
PINUS SYLVESTRIS

A NEW MEMBER OF THE CAYTONIALES

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(With Plates IV and V and 12 figures in the text)

INTRODUCTION

THE class Caytoniales consists primarily of two types of fruits, *Caytonia sewardi* and *Gristhorpia nathorsti* from the Middle Jurassic of Yorkshire; there is evidence suggesting that the associated leaves (*Sagenopteris phillipsi*) and the microsporophylls (*Antholithus arberi*) belong to one or perhaps both of these plants (Thomas, 1925). Subsequently some seeds and anthers of the *Caytonia* type were found associated with *Sagenopteris nilssoniana* in Greenland (Harris, 1926*b*), and a specimen of *Gristhorpia nathorsti* was found associated with *Sagenopteris goepperiana* in Sardinia (Edwards, 1929). The present specimens were obtained from two localities in Scoresby Sound, East Greenland (lat. 70° N.). The beds in these two localities are of the same age and belong to the very base of the Lias, which in its plant-bearing facies was formerly included in the Rhaetic. Some of the specimens were collected by Dr N. Hartz in 1902 (Amdrup expedition); the majority by me in 1926–7 (on Lauge Koch's expedition).

DIAGNOSIS. *Caytonia thomasi* sp.nov.

Closed, dorsiventral fruits 5–7 mm. wide, 2–3 mm. long, borne on slender stalks. Stigma close to the stalk, 2–3 mm. wide and very short and marked by about 30 vertical furrows. Surface of fruit and of fruit-stalk bearing hairs composed of about three cylindrical cells surmounted by a spherical cell. Epidermal cells of fruit with straight walls. Fruit containing about 30 seeds; seeds pendulous, orthotropous, ovoid, 1·4 mm. long, surface smooth, integument thick, mainly composed of stone cells (with resistant cell contents), cells lining micropylar canal isodiametric. Inner cuticle of integument very delicate, showing elongated cells; cuticle of nucellus fairly thick, showing outlines of cells 120 μ long × 30 μ broad over most of its surface but smaller near the base and apex; cuticle of megaspore soft, showing finely granular structure.

DETAILED DESCRIPTION

Fruit and seed. Most of the fruits were obtained by dissolving in hydrofluoric acid a single very small fragment of shale collected from Cape Stewart. The arrangement of the fruits was noted before the rock was quite dissolved, and it was obvious that they had all been borne on a single main stalk, but this stalk was not actually found. A few other fruits were obtained by the maceration of other pieces of shale from the same bed.

The type specimen (Pl. IV, figs. 17–19; Pl. V, fig. 7) has a short stalk; the stalk was broken off in the other figured specimen (Pl. IV, figs. 15, 16). The "stigma" forms a conspicuous ridge crossing at the top of the stalk on one side of the fruit; it is recognisable in every specimen and serves to distinguish the stigma-bearing from the opposite or stalk side of the fruit. The surface of the fruit shows oval bulges caused by the seeds within, the bulges are more conspicuous on the stigma-bearing side. The dimensions of the fruits vary only slightly, the two specimens figured represent exteme forms.

On maceration (strong HNO_3 + $KClO_3$ followed by NH_4OH) the main part of the fruit wall dissolves, leaving its cuticle, which forms a closed sac. On the stalk side the cuticle shows polygonal epidermal cells, on the stigma-bearing side and on the stalk the cells are more or less square and are arranged in longitudinal rows. The cuticle over most of the fruit is rather thin, but on the stigma it is very thick indeed. The fruit and its stalk bear short filamentous hairs which are usually composed of three cells, the apical one being swollen (Pl. IV, fig. 8). The apical cell of the hair has a good deal of dark contents which are dissolved on maceration, its wall is less thickly cutinised than that of the lower cells and is often more or less destroyed when the specimen which has been macerated in acid is placed in alkali. Stomata are very rare, but some were probably found on the stigma-bearing surface (Text-fig. 3); it is possible, however, that these are not stomata but the basal cells of hairs.

The stigma in the unmacerated seed is a thin flange standing out from the stalk, its free margin is bent back towards the fruit, and is marked with about thirty ridges which end in very small teeth (Pl. V, fig. 7). On maceration it is found to consist of several layers of cuticle, some very thick, some thin folded over one another; the exact relation of these cuticles to one another and to that of the stalk could not be determined with certainty in the small number of fruits available. The most prominent layer of cuticle is divided into

thick and thin bands (Pl. V, figs. 2, 3) (corresponding to the ridges or teeth seen before maceration), the cells forming the thick bands consist of a few rows running longitudinally down the middle of the band and of others diverging from these at an angle of about 40° at the sides, thus forming a herring-bone pattern.

The cuticle belonging to the interior of the fruit was not definitely recognised, but a few strips of very delicate cuticle were obtained which may well be this layer.

All the seeds are flattened, most of them being compressed laterally, but a few, chiefly of those found inside fruits, have been compressed obliquely or vertically, and as these seeds are almost circular it seems certain that the seed must have originally been circular in cross-section.

A large number of seeds were obtained from fruits by maceration, or by chipping away the fruit wall, and several hundred isolated seeds were also obtained by dissolving the *Caytonia*-bearing shale in hydrofluoric acid. The isolated seeds (Pl. IV, figs. 1–4) agree precisely with those found inside fruits and certainly belong to the same species. The seed is smooth and ovoid, the chalaza is at the sharp end and the micropyle at the blunt end. Occasionally a small spike of tissue projects from the chalaza; this, on maceration, proves to be quite distinct from the rest of the seed and may be a remnant of the funicle (Pl. IV, figs. 2, 6).

On maceration the seed swells somewhat and the greater part of its substance dissolves, leaving the following resistant parts:

> Outer and inner cuticles of the integument.
> Outer and inner "spicules" of the integument.
> Cuticle of the micropylar canal.
> Cuticle of the nucellus.
> Megaspore membrane.

The outer cuticle of the integument is delicate and only shows the walls of cells faintly. The cells are slightly elongated, and their walls often appear to be finely dotted (Text-fig. 5). Near the micropyle and also near the chalazal end of the seed, the cells are smaller and isodiametric and their lateral walls are much more strongly marked.

The "spicules" which loosely fill the space between the inner and the outer cuticles of the integument are internal casts of thick-walled, pitted cells, the walls of which are seen in partly macerated sections (Text-fig. 8) but which have been dissolved by more complete maceration. The outer spicules (Text-fig. 6) are situated close to the

integument cuticle and are often found sticking to it. They have not been previously observed, but belong to a layer of rectangular cells, which is the same as the "palisade layer" of *Caytonia sewardi*.

The inner "spicules" (Pl. IV, fig. 12), which are like those of *C. sewardi*, are derived from elongated cells. They occur in all parts of the seed but are most numerous at the chalazal end. The spicules are composed of a substance less resistant to maceration than is the cuticle of the nucellus and integument, for after a week's maceration they tend to liquefy.

Some seeds were partially macerated by the use of a somewhat diluted acid solution, which results in a considerable swelling of the seed coat but only partial solution of its coaly substance; they were then dehydrated, embedded in paraffin and microtomed. The sections (Text-figs. 7, 8) throw some light on the structure of the integument. The outermost layer is a cuticle which had usually separated more or less from the rest. About half of the thickness of the integument is made up of a layer of rectangular cells with exceedingly thick walls—the "palisade layer"; then come several layers of smaller cells—the "fibrous layer," to which the inner spicules evidently belong. Within this there are several layers of cuticle, the details of which were not satisfactorily made out. The cuticles of the interior of the seed extend practically up to the fibrous cells at the flattened margins of the seed (Text-fig. 7); had there been originally a considerable thickness of delicate tissue inside the integument, the inner cuticles would have been expected to stop short of the fibrous layer at the margins even if the delicate tissue had been so crushed that its cells became unrecognisable.

The inner cuticle of the integument (Pl. V, fig. 8) is exceedingly delicate, and traces of it are sometimes found investing the nucellus after the "spicules" have been removed. It appears to be continuous above with the cuticle of the micropylar canal, and shows traces of the outlines of elongated cells of the same shape as those on the outside of the integument.

The micropylar canal seems to be a rather broad funnel-shaped tube and is lined by small cells with prominent outlines (Pl. IV, figs. 10, 11; Pl. V, fig. 4). At the base of the canal the cuticle becomes more delicate.

In rather more than half of the seeds with well-preserved micropylar canals, it is possible to detect pollen grains in the interior of the canal (Pl. V, fig. 4). In most cases these grains are of the *Antholithus arberi* type, but a few other spores were also found.

The chalaza is figured on Pl. V, figs. 5, 6, and Harris (1926 b), Pl. VIII, fig. 7, where, owing to the inadequate material, it was mistaken for the micropyle. There is a great accumulation of the spicules (fibrous cells) round the chalaza, and some of these form a compact strand running from the base of the nucellus to the funicle. The tissue sometimes found attached to the hilum and considered to be the remains of the funicle shows no structure beyond a faint indication of cells (Pl. IV, fig. 6).

The cuticle of the nucellus closely invests that of the megaspore and can only be separated from it after long maceration. It is thicker and tougher than the other cuticles and shows the outlines of cells very clearly indeed. In the micropylar and chalazal regions the cells are isodiametric (Harris (1926 b), Text-fig. 12 G, Pl. VIII, fig. 8), elsewhere they are elongated (Pl. IV, fig. 9). In some specimens the sides of the cells are slightly sinuous, but this is possibly only an artefact caused by maceration. At the base of the micropyle the nucellus cuticle often appears to come to an end, leaving a very small hole; but in other seeds it seems to be continuous over the top of the megaspore. At the chalaza, the nucellus cuticle comes to an abrupt end, leaving a hole 120 μ in diameter (Pl. V, fig. 4) (in seeds which had been compressed vertically this hole is seen to be round). It is probable that the inner cuticle of the integument is continuous with that of the nucellus at the chalaza, but this could not be demonstrated.

The megaspore membrane is a cuticle of very varied thickness in different seeds. It shows no indication of cells, but is marked with innumerable minute granules or pits (too small to be seen in the photograph on Pl. IV, fig. 9). In many seeds the megaspore membrane has folded transversely—this is no doubt an effect caused by preservation, but is nevertheless characteristic of this species of seed.

In about a fifth of the seeds examined, the megaspore projects upwards a short distance in the micropylar region (Pl. IV, fig. 10), forming a "plinth" comparable with that seen in *Gristhorpia*. The "plinth" is closely invested by a cellular cuticle which is probably that of the nucellus.

The reconstruction given in Text-fig. 9 is based on the evidence given above. The thickness of the various cuticles is exaggerated, but apart from this it is supposed to be drawn to scale. There is objective evidence from the cuticles or from sections of the existence of all the layers of cells shown, except the inner layer of the nucellus which is entirely imaginary.

Mic.

O.ep.

Pal.

Fib.

In.ep.

Nuc.

Meg.
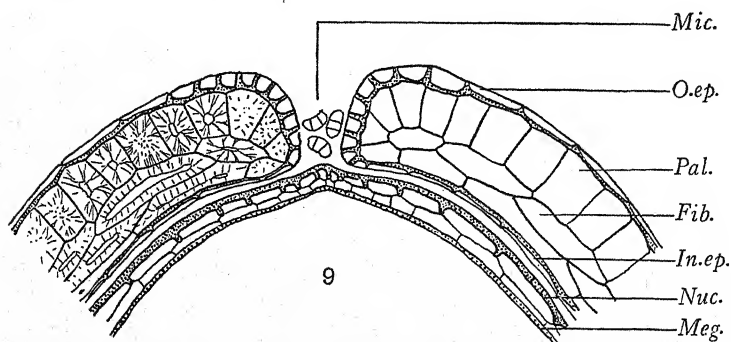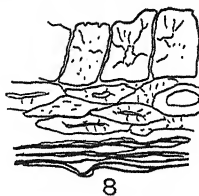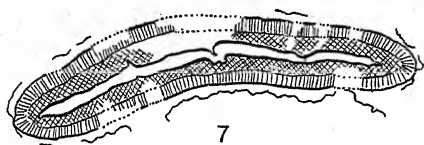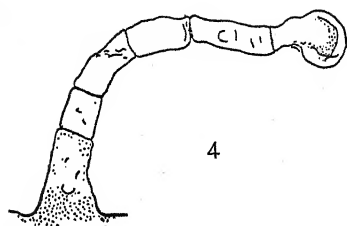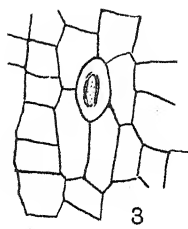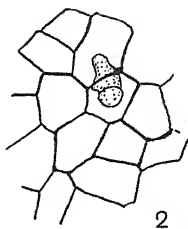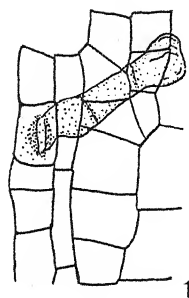
Male organs. The specimens are: some nearly ripe "anthers"; some anther-bearing stalks; and numerous free pollen grains, all of which specimens were obtained by the maceration and solution of rock in bulk either by hydrofluoric acid or by the method described by Harris (1926 *a*).

Twelve well-preserved anthers were found; each anther consists of a delicate cuticle enclosing four elongated masses of pollen grains. The pollen masses are arranged radially and adhere to one another along the middle of the anther, but can be separated after maceration, and after longer maceration some of the pollen grains can be detached from the masses. Typical specimens of anthers are shown in Pl. IV, figs. 13, 14, and in Harris (1926 *b*), Pl. VIII, fig. 4. The length of the anther varies from 2 to 8 mm., but its breadth is more constant. The cuticle is delicate and only shows faint indications of elongated cells.

A fragment of an anther-bearing organ is shown in Text-fig. 12 and others are shown in Harris (1926 *b*), Text-fig. 12 *b*. They consist of a main axis with lateral branches which divide and terminate in rounded heads. The cuticle shows the walls of elongated cells and of some oval cells which are possibly bases of hairs. The head of the branch is composed of shorter cells than the rest, and on one side the cuticle is torn away leaving an irregular or round hole, where it is believed that an anther was attached.

Pollen grains of the *Antholithus arberi* type occur in abundance in the rock with *Sagenopteris* and often cover the upper surface of *Sagenopteris* leaves (Harris (1926 *b*), Pl. VIII, figs. 5, 9). The size and

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Text-figs. 1–9. 1. *Caytonia thomasi*, cuticle of stigmatic side of fruit, showing a hair. Slide 682, × 200. 2. Cuticle of non-stigmatic side of fruit, showing a unicellular hair. Slide 682, × 200. 3. Cuticle of ventral surface of fruit, showing a stoma (or possibly a hair-base). Slide 682, × 200. 4. *Sagenopteris nilssoniana*, cuticle of large hair on margin of petiole. Slide 676, × 200. 5. *Caytonia thomasi*, outer cuticle of integument of seed showing moniliform outlines. Slide 693, × 200. 6. Outer spicules of seed adhering to outer cuticle of integument. Slide 695, × 200. 7. Semi-diagrammatic representation of section of seed; outer wavy line = outer cuticle of integument, layer shaded transversely = palisade, layer cross hatched = fibrous layer, innermost line = crushed cutinised membranes. Dotted parts are restored. Slide 717, × 50. 8. Part of one of these sections showing the palisade layer above, the fibrous layer (middle) and the crushed cuticles of the interior of the seed (below) (somewhat diagrammatic), × 200. 9. Restoration of micropyle of seed: *Mic.* = micropyle with pollen grains; *O.ep.* = cuticle of outer epidermis of integument; *Pal.* = palisade layer of stone of integument; *Fib.* = fibrous layer of stone; *In.ep.* = cuticle of inner epidermis of integument; *Nuc.* = cuticle of nucellus; *Meg.* = cuticle of megaspore.

shape of the grains is almost constant; those in which the wings are situated slightly above the middle of the grain are probably viewed from the side, and those in which they appear more symmetrical are probably viewed from above or below (Text-fig. 11).

This male organ is too incompletely known for it to be possible to distinguish it from *Antholithus arberi*; I have therefore refrained from giving it a new specific name.

The leaf. Sagenopteris nilssoniana (= *S. rhoifolia*) is a rather well-known species; it has been described by Halle (1910) and by many other authors whom Halle cites. It possesses a petiole bearing four leaflets, a typical leaflet of which is shown in Pl. IV, fig. 7. The size of the leaflets varies greatly (the largest Greenland specimen is 14 cm. and the smallest 2 mm. long). The smallest leaves sometimes bear three or more pairs of pinnae and they are less than usually crowded (Harris (1926 b), Text-fig. 12 A). The cuticle (which will be described in a future paper on the Greenland flora) has a more constant structure and has a greater diagnostic value than the leaf form.

Filamentous hairs with spherical apical cells are found on the petioles, margins and main veins of the leaves, especially of the smaller leaves. These hairs are only seen well in specimens obtained by dissolving the rock, since when the leaf is picked off the rock the hairs are left behind. The hairs may be slightly larger or smaller than those of the fruit, but are of exactly the same type (Text-fig. 4).

The reference of the different organs to the one species

The organs described here agree in their main features with those described by Thomas from Yorkshire, *Caytonia thomasi* with *C. sewardi* and *Gristhorpia nathorsti*, the male organ with *Antholithus arberi* and the leaf with *Sagenopteris phillipsi*. Part of the interest of the Greenland material lies in the support it gives to Thomas's view that all these organs belong to one group of plants.

Thomas (1931) has restated his evidence for referring the above-mentioned Yorkshire leaves and male organs to *Caytonia* and *Gristhorpia*. Briefly, this depends on their close association in the field and on structural agreement between them, especially the cells of the main stalks or petioles, which are of very similar shape. The evidence is complicated by the fact that there are two members of the Caytoniales—with obviously different fruits occurring in the same bed, but the leaves and male organs belonging to them cannot so easily be divided into two groups. It seems probable that the male organ of only one—*Gristhorpia*—is known, while the leaf is really

two species distinguishable by the structure of their cuticles, one resembling *Caytonia* and one *Gristhorpia*.

In Greenland, this complication does not arise because in the beds with which we are concerned there is only one species of fruit, one of male organ and one of leaf. These organs are closely associated in each of two localities 15 kilometres apart, but in beds of the same age.

The leaf, *Sagenopteris nilssoniana*, is a rather rare species in Greenland, being known from only five localities, but it is abundant in certain layers of rock in two of these five, and it is here, and here only, that the reproductive organs were found. The two localities have only one other seed plant in common—*Pterophyllum subaequale* —a member of the Bennettitales.

The cuticle of the fruit wall of *Caytonia thomasi* is composed of cells resembling those of the upper side of the leaf *Sagenopteris nilssoniana*, and it possesses hairs which are just like those on the petioles, margins and main veins of *S. nilssoniana*; as this type of hair has not been met with in any other Greenland plant it provides strong evidence that the leaf and fruit possessing it belong to the same species. There is thus strong evidence from association and from agreement in structure that *Caytonia thomasi* belongs to *Sagenopteris nilssoniana*.

The association of the anthers with the *S. nilssoniana* is just as marked as that of the fruits. There is not, however, the same evidence of agreement in structure. Perhaps because they are fragmentary, the male organs show no specific feature of agreement with the leaf or fruit, though they do not show any point of difference. They agree, however, with *Antholithus arberi*, a better known fossil, which does agree in the shape of its cells with *Gristhorpia* (Thomas, 1925), and on this evidence, combined with that of association, it is referred to . *Sagenopteris nilssoniana*.

The pollen grains obtained from the anthers are characteristic and easily distinguishable from those of other Greenland plants. Like Thomas, I succeeded in finding the pollen grains on the stigma and on other parts of the fruit.

This evidence of pollination for referring male and female organs to the same species, unless in some way supported, is known to be of only slight value (Sahni, 1915); in *Caytonia thomasi* at least ten other types of pollen grains or fern spores were found on the fruits, and the *Antholithus arberi* type was by no means the commonest; in the micropyles of the seeds, though the *A. arberi* type of pollen was actually the commonest, a few other types of spores were found.

MORPHOLOGY

Nothing is known about the fruit-bearing stalk of *Caytonia thomasi*; the morphology of the infructescence of *C. sewardi* and *Gristhorpia nathorsti* has been discussed as fully by Thomas (1925, 1931) as our knowledge allows.

The fruit of *Caytonia thomasi* very closely resembles that of the other two species, but the discovery of pollen grains in seeds inside the fruits makes necessary a revision of our views on its structure. The almost unlimited supply of seeds available, which enabled me to sacrifice as many as I wished to clear up a single point, has also resulted in the recognition of several other new features, which are probably not peculiar to this species, but shared by the others.

The following facts must be taken into consideration in discussing the pollination of *C. thomasi*:

(1) The considerable majority of well-preserved seeds of *C. thomasi* possess pollen grains (chiefly but not entirely of the *Antholithus arberi* type) in their micropyles. The fruit must therefore have been open at the time of pollination.

(2) No specimen of a fruit contains any rocky matrix. The fruits must therefore have been closed or almost closed at the time they were shed and preserved.

(3) The outside of the fruit (including the stigma) is frequently covered with spores and pollen grains in considerable variety and profusion. Practically no pollen grains (apart from those in micropyles) occur in the interior of the fruit—whether on the fruit wall, or loose or on the integuments of the seeds. (Some fruits were macerated specially to confirm this point.) This shows that there must be some special structure in the fruit which directs the pollen grains to the micropyles, for without it the majority of the pollen grains would almost certainly be scattered in the fruit, chiefly round the bases of the seeds.

The following observations are of less certain interpretation, but the hypothesis framed below is based partly on them.

(4) The stigmas in all species are marked with dark and light bands—about 30 in *Caytonia thomasi*, 14 in *Gristhorpia nathorsti* and 10 in *Caytonia sewardi*; these numbers approximately equal the number of seeds in the fruit of each species. The bands are evidently definite structural features and are caused by a difference in the arrangement of the cells of the stigma and the thickness of their cuticles.

(5) In *C. sewardi*, Thomas observed delicate strands of cutinised membrane connecting the micropyles of the seeds with the stigma of the fruit (these strands are also discussed on p. 109).

The interpretation suggested for observation (4) is that the dark and light bands represent minute canals running through the stigma, open at the time of pollination, but probably occluded in the ripe fruit. The strands of cuticle (observation (5)) are regarded as the continuation of the stigmatic canals to the micropyles of the seeds.
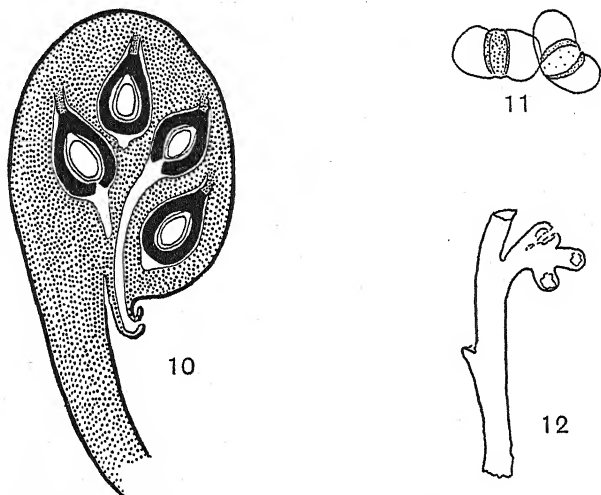
Text-figs. 10–12. 10. Restoration of fruit in longitudinal section. Fruit wall dotted, stone of seed solid black, cuticles continuous black lines. The inner epidermis of the fruit forms a pocket round each seed connected by a narrow channel with the stigma; three of the seeds are in non-median section and the channel is not seen. 11. A pair of pollen grains sticking to the upper cuticle of a leaf of *Sagenopteris nilssoniana*. The left-hand grain is probably viewed from above, the right-hand one from the side. Slide 610, × 400. 12. An anther-bearing organ, with a three-lobed branch, the cuticle of each lobe being torn where an anther was probably attached. Slide 607, × 10.

The hypothesis put forward is embodied in the restoration shown in Text-fig. 10. The fruit is supposed to be essentially a hollow cup-shaped organ, bent back against its stalk after the manner of an anatropous ovule. The lip of the cup is somewhat contracted and is flattened until the two sides have met and have closed it, except for a series of narrow passages. The seeds are attached in rows to the walls of both sides of the fruit, the inner part of the fruit wall is supposed to be very thick and fleshy, embedding the seeds in closely fitting pockets and nearly obliterating the cavity, though leaving a

series of narrow canals which extend from the canals through the stigma to just above the micropyles of the seeds.

These canals are supposed to be lined with the inner epidermis of the fruit possessing a very delicate cuticle; between the canals the two faces of the inner epidermis are pressed against one another and have no recognisable cuticle.

Pollination is supposed to be caused by the secretion by the ovules of a liquid which passes down the canal to the stigma where it catches wind-borne pollen grains, and then is absorbed by the ovules, carrying pollen grains up the channels and into the widely open micropyles.

It should be possible to demonstrate, or to disprove the existence of canals through the stigma by a closer investigation of its structure, but from material such as is at present available it seems unlikely that we shall be able to obtain entirely satisfactory information about the delicate inner tissues of the fruit.

The type of pollination which is here supposed to occur in *C. thomasi* where pollen is conveyed along open channels to the ovules is obviously different from that met with in the Angiosperms, where the pollen grains remain on the stigma and send pollen tubes down the stylar canal or through the stylar tissue to the ovules.

There is virtually no evidence to show what was the pollination mechanism of primitive Angiosperms (such variations as occur in the stigma, e.g. in *Zannichellia* or *Reseda*, being no doubt secondary), but it seems by no means unlikely that it may have been similar to what is postulated for *Caytonia*. Possibly the style was originally an open canal along which the pollen was conveyed (on a drop of fluid) to the ovules; a later stage would be the germination of the pollen grain before it reached the micropyle, at first no doubt at the bottom of the stylar canal, then at its middle and then at its top. The final change to occur—the closure of the stigma—makes it impossible for the pollen to pass down the stylar canal.

The Seed

The seed of *Caytonia thomasi* is so like that of *C. sewardi* that there can be no doubt that it shares the new features found in *C. thomasi*; indeed I have been able to recognise practically all of them, though not as clearly as in *C. thomasi*, which is more favourable to study, having slightly thicker cuticles.

The epidermis or "blow-off layer" of the integument of *C. thomasi* seems to be thinner than that of *C. sewardi* and I have been

unable to make out whether the cuticle which invests the seed belongs to its inner or to its outer walls. In *C. sewardi* it has been shown by sections that it certainly belongs to the inner wall, and I therefore regard it as the inner wall in *C. thomasi*. Indirect evidence in favour of this view is provided by the fact that in *C. thomasi* the internal casts of the palisade layer are often found adhering to the investing cuticle of the seed.

The transition from the apical part of the integument cuticle, where the cells are small and with well-marked outlines, to the part round the sides of the seed, where the cells are larger and have faint outlines, is often less abrupt than in *C. sewardi*. This suggests that there is a single epidermis over the whole of the integument which becomes modified as it approaches the micropyle rather than being replaced by another layer of cells as Thomas has tentatively suggested (1925, p. 325, Text-fig. 9).

I have not been able to recognise the cutinised strands which Thomas found in *C. sewardi* running from the micropyle of the seed to the stigma. Thomas suggested that these strands might be the remains of pollen tubes or of a delicate "beak" formed by an inner integument. The latter of these suggestions can, I think, be ruled out because there is not only no cutinised remains of such a beak in isolated seeds, but there is no break in the cuticles at the micropylar end of the seed—a fact which shows that they are complete. The alternative suggestion is put forward that the strands are the cuticle of the inner epidermis of the fruit wall and that they played an important part in pollination (p. 108).

The plinth, where it is present, is composed of a cellular membrane (probably an upward extension of the nucellus) and encloses a certain amount of structureless material which is probably the megaspore membrane. It is a minute and variable feature and by no means fully understood, and little weight can be put upon its apparent agreement with the "plinth" of Pteridosperm seeds.

The spicules of *C. thomasi* are usually a little better preserved than those of *C. sewardi*, but are otherwise similar; the outer "spicules"—that is the casts of the "palisade cells"—had not been described in *C. sewardi* but are nevertheless discernible in certain preparations. There can be no doubt that Thomas's interpretation of the "spicules" as internal casts of thick-walled cells is correct, the sections show this conclusively; but it is certainly extraordinary that the contents of a cell should survive fossilisation and maceration better than its wall. No other fossil seed I have examined has yielded

"spicules," though most of them have thick-walled cells in the testa; the presence of spicules in *Caytonia* must result from some peculiarity in the chemical nature of the original contents of the cells of the testa rather than from a structural peculiarity.

The delicate inner cuticle of the integument had not been previously recognised.

The membrane which Thomas termed the megaspore has been shown in *C. thomasi* to be composed of two; the outer, the nucellus cuticle showing cell outlines, the inner, the megaspore membrane showing no cellular structure but only minute granular pits. The megaspore presents no unusual features, but the comparison of the nucellus cuticle with that of other seeds gives results of considerable interest.

In the Gymnosperm seeds I have examined (Cycads, Conifers and Ginkgo), where the nucellus is "free" it has a definite epidermis and cuticle and so has the inner surface of the free part of the integument. Where the integument is "fused," the epidermis and cuticle of the nucellus joins that of the integument and there is naturally no cuticle below this point.

In fossil Gymnosperm seeds, I have frequently been able to find a cuticle situated deep in the wall of the seed which shows the outlines of two sets of cells superimposed on one another. Occasionally this membrane can be separated into two, and I believe it to be composed of the cuticles of the nucellus and of the inside of the integument stuck together (Harris (1926 b), pp. 120, 127; Harris (1932), pp. 53, 67). In other fossil seeds there is no cuticle at all in this position, probably because the nucellus and integument were not "free." In no Gymnosperm seed examined have I found a fairly thick cuticle on the nucellus accompanied by a mere vestige only of a cuticle, as in *Caytonia*, on the inner side of the integument facing it.

The seeds of certain Angiosperms, however, show this feature. The seed of *Cucurbita pepo* has a cuticle on the outside of the integument and also quite a thick cuticle on the outside of the nucellus, which is the green membrane inside the testa. This membrane shows the outlines of the epidermal cells very clearly and is thus like that seen in *Caytonia*; the main point of interest is that the epidermis of the inner face of the integument has practically no cuticle at all; and if a *Cucurbita* seed were fossilised its structure, as revealed by maceration, would probably seem to be very similar to that of *Caytonia*.

Thomas (1925, 1931) has suggested that the seed of the Caytoniales shows important points of agreement with the seeds of various Gymnosperm families—particularly the Cycads, Pteridosperms and Bennettitales—in the comparative simplicity of its integument; it seems to me, however, rather to resemble the Angiosperms and to differ from the Cycads and Pteridosperms. Our knowledge of the cutinised parts of the Bennettitalean seed is hardly adequate, as yet, for any comparison to be made, though the small size of the seeds in the Bennettitales makes it quite likely that some of them might have as simple integuments as *Caytonia*.

The fragments of the anther-bearing organ so far obtained show nothing that was not already known in *Antholithus arberi (Gristhorpia)*. The anthers, however, afford strong support to the view expressed by Thomas (1925, Text-fig. 10 A) that the four pollen sacs are of equal size and are disposed radially. This seems a most important difference from the Angiosperm stamen, where I believe that such arrangement is unknown, the great majority of stamens having a connective with two lateral thecae, each with two pollen sacs.

Comparison.

	Caytonia sewardi	*Caytonia thomasi*	*Gristhorpia nathorsti*
Fruit	Fruit round	Fruit broader than long	Fruit round
	Stigma 0·5 mm. long × 0·5 mm. wide (approx.)	Stigma 0·5 mm. long × 2·5 mm. wide (approx.)	Stigma 0·8 mm. long × 1·5 mm. wide (approx.)
	Striations of stigma inconspicuous	Striations of stigma conspicuous	Striations of stigma conspicuous
	Cuticle of fruit thick	Cuticle of fruit thin	Cuticle of fruit thick
	Cell outlines thick, sinuous. No hairs	Cell outlines thin, straight. Hairs on fruit wall	Cell outlines thick, straight. No hairs
Seed	8–10 seeds per fruit	20–30 seeds per fruit	10–12 seeds per fruit
	Testa thick, fibrous, hence: seeds cause bulges in fruit	Testa thick, fibrous, hence: seeds cause bulges in fruit	Testa thin, hence: seeds do not cause bulges in fruit
	Seeds known to be arranged in rows	Seeds known to be arranged in rows	Arrangement of seeds unknown
	On maceration yields "spicules"	On maceration yields "spicules"	On maceration yields no "spicules"
	Surface pitted	Surface smooth	Surface smooth
	Cells of micropyle tube isodiametric	Cells of micropyle tube isodiametric	Cells of micropyle tube elongated
	No "plinth"	"Plinth," occasionally present	"Plinth" usually present

The differences between the fruits and seeds of the three species of the Caytoniales at present known are set out in the table; on the whole the fruit of *Caytonia thomasi* agrees better with that of *Gristhorpia*, its seed with *Caytonia sewardi*, and as this is perhaps the more important I have included it in *Caytonia*. In whichever genus it is placed it removes some of the differences between *Caytonia* and *Gristhorpia* which are given by Thomas (1925, p. 326), the only important one remaining is that the testa of *Caytonia* is thick and its cells have internal casts (spicules), while that of *Gristhorpia* is thin and its cells yield no spicules. I am insufficiently acquainted with the seeds of recent plants to know whether this is a difference of generic or only of specific value. Should it eventually be considered advisable to unite *Caytonia* and *Gristhorpia*, the name *Caytonia* would seem preferable, as the structure of the fruits and seeds in *C. sewardi* is better known than in *Gristhorpia nathorsti*.

The male organ of *C. thomasi* has pollen grains which seem to be very slightly larger than those of *Antholithus arberi*, the anthers are perhaps of less uniform length and borne in less crowded clusters. It is, however, not well enough known for full comparison with *A. arberi*.

The leaf of the Yorkshire Caytoniales *Sagenopteris phillipsi* is, as Thomas indicated (1925, p. 333), really referable to two species distinguishable from one another by their cuticles. The upper epidermis of one has sinuous-walled cells like *Caytonia sewardi*, of the other has straight, very thick-walled cells like *Gristhorpia nathorsti*. The upper cuticle of *Sagenopteris nilssoniana* has straight thin-walled cells which distinguish it from the Yorkshire species. I intend to compare these leaves more fully in a future paper on the Greenland flora.

SUMMARY.

1. A new species of fossil fruit, *Caytonia thomasi*, is described from the basal Liassic rocks of East Greenland.

2. A male organ resembling *Antholithus arberi* is described and referred to *Caytonia thomasi*.

3. The well-known leaf, *Sagenopteris nilssoniana*, is referred to the same species as *Caytonia thomasi*.

4. Pollen grains have been found in the micropyles of the seeds showing that in pollination *C. thomasi* is essentially gymnospermous. The bearing of this fact on the probable structure of the fruit and on the comparison with the Angiosperms is discussed.

5. Certain new features shown by the seed of *C. thomasi* (but probably shared by the other species) are described and discussed.

6. *Caytonia thomasi, C. sewardi* and *Gristhorpia nathorsti* are compared with one another.

In conclusion I wish to express my gratitude to Dr H. Hamshaw Thomas for assisting me by lending his preparations of the Caytoniales and for discussing the new features which were found as a result of the present investigation.

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EXPLANATION OF PLATES

PLATE IV

Figs. 1–4. *Caytonia thomasi*, isolated seeds. The micropylar end points upwards. The seed shown in fig. 2 has part of the funicle attached to the chalaza. × 10.

Fig. 5. *Caytonia thomasi*, macerated seed, showing the cuticle of the integument; thickened near the micropyle and the chalaza; and the megaspore-nucellus membrane. Slide 691, × 20.

Fig. 6. *Caytonia thomasi*, seed shown in fig. 2 macerated, showing the tissue of the funicle attached to the chalaza. Most of the integument cuticle has been torn away. Slide 692, × 20.

Fig. 7. *Sagenopteris nilssoniana*, a segment of a normal sized leaf (immersed in xylol to show the veins). Specimen 1211, × 1.

Fig. 8. *Caytonia thomasi*, fruit, hairs on fruit stalk, only partially macerated and showing dark contents in terminal cells. Slide 686, × 100.

Fig. 9. *Caytonia thomasi* seed, cuticles of nucellus and of megaspore separated from one another by long maceration. Slide 697, × 100.

Fig. 10. *Caytonia thomasi* seed, micropylar region, showing integument cuticle with smaller cells near the top; the confined cuticle of the nucellus and megaspore and the "plinth". Slide 691, × 100.

Fig. 11. *Caytonia thomasi* seed, micropyle of another seed in which scarcely any "plinth" is seen. The integument has broken away a short distance from the apex. Slide 694, × 100.

Fig. 12. *Caytonia thomasi*, seed, "spicule" (internal cast of thick-walled cell) from integument. Slide 689, × 250.

Figs. 13, 14. *Antholithus arberi* type of male organ; the two sides of the same stamen showing the four anther sacs. Slide 700, × 10.

Figs. 15, 16. *Caytonia thomasi*, fruit. Two views of the stigmatic side of the same fruit lit from the top in 14, the bottom in 15. The stigma is at the base, the stalk is broken away. Slide 683, × 4.

Figs. 17, 18, 19. *Caytonia thomasi*, fruit. Type specimen. Figs. 17 and 18 show the stigmatic side, lit from the top left corner in 17, the bottom right corner in 18. Fig. 19 shows the non-stigmatic surface (lit from the top left corner). Slide 681, × 4.

The figures are untouched photographs; the *Sagenopteris* leaf was obtained from Dinosaur River, *Marattiopsis* Bed, all the other specimens from Cape Stewart, *Anomozamites hartzi* Bed.

PLATE V

Fig. 1. Lower edge of stigma, viewed from the side facing the stalk. The uppermost layer of cuticle is broken at the point *x* and bent upwards at the point *z*; a deeper layer is bent upwards at the point *y*. Slide 688, × 250.

Fig. 2. The whole of a stigma, the free edge facing the left. It is curved, largely owing to unequal swelling in maceration. Transverse striations can be seen in places. Slide 696, × 16.

Fig. 3. Upper edge of stigma, viewed from side away from stalk, showing two of the locally thickened regions of the cuticle. Slide 687, × 250.

Fig. 4. Micropylar region of seed. The cuticle lining the micropyle appears as a cellular tissue; at the base of the micropyle is seen a winged pollen grain (*P*). *N* = the nucellus enclosing the megaspore. Slide 711, × 250.

Fig. 5. Chalaza of seed. *N* = nucellus enclosing the megaspore, *S* = space inside cuticle of integument, *I* = outer cuticle of integument, *H* = hilum. Slide 691, × 100.

Fig. 6. Chalazal region of seed from which all tissues outside the nucellus cuticle have been removed. Note the opening in the base of the nucellus. Slide 612, × 100.

Fig. 7. Part of fruit, showing the stigma. Slide 681 (type specimen), × 16.

Fig. 8. Side of seed; the outer cuticle of the integument and the spicules have been removed, fragments of the delicate inner cuticle of the integument *I* are seen. *N* = outer edge of cuticle of the nucellus beyond the edge of the megaspore, *M* = cuticle of integument enclosing the megaspore and hence appearing darker than *N*, the nucellus alone. Slide 685, × 250.

All the specimens figured were obtained from Cape Stewart, *Anomozamites hartzi* Bed, except those shown in Pl. V, fig. 6 and in Text-figs. 11 and 12, which were obtained from Vardekløft, *Podozamites agardhianus* Bed, and that shown in Pl. IV, fig. 7, which was obtained from Dinosaur River, *Marattiopsis* Bed.
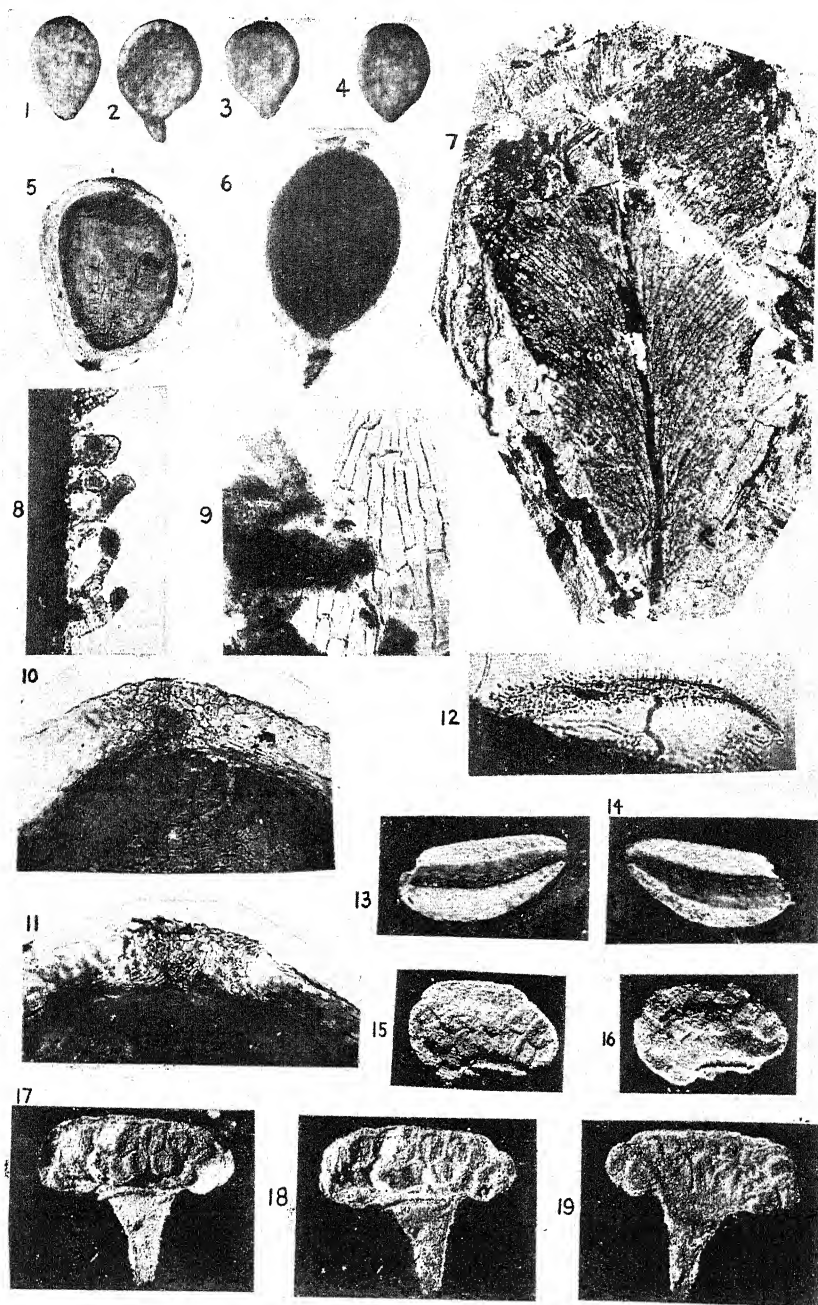
HARRIS—A NEW MEMBER OF THE CAYTONIALES

NON-SYMBIOTIC DEVELOPMENT OF SEEDLINGS OF *CALLUNA VULGARIS*

By LEWIS KNUDSON

(With Plates VI and VII)

RAYNER (3, 4, 5, 6) for a considerable number of years has investigated the heather *Calluna vulgaris* and particularly the relation of the root fungus to *Calluna vulgaris*. The fungus component was described by Rayner as *Phoma radicis Callunae,* and the distribution of the fungus in its host determined. As a result of various studies Rayner came to the conclusion that seedling development of *Calluna vulgaris* is dependent on infection of the seedlings by *Phoma radicis Callunae*. According to Rayner "germination takes place on the 18th to 21st day as with untreated seeds. The minute embryo absorbs food material from the endosperm, and increases rapidly in size to form a normal seedling consisting of a short hypocotyl, bearing two cotyledons, and terminated at either end respectively by plumular bud and short blunt radicle. At this stage there is a pause in development, easily discernible in *seedlings germinating* under natural conditions; in those produced from properly sterilised seeds, root development is inhibited and this is followed by arrest of growth and the inevitable symptoms of malnutrition" (Rayner (6), p. 378).

In 1929 I (1) presented experimental evidence demonstrating that seedling development occurs without the intervention of the fungus. Rayner's solution A was used as a nutrient medium with the addition of 1·5 per cent. agar. The reaction was adjusted so that a series of pH values was obtained. In the first experiment reported a fungus contamination occurred which was identified as a species of *Alternaria*. This fungus developed from bits of floral tissue introduced with the seed, but no cells of the roots were found infected. In Exps. 2 and 3 the cultures were free from micro-organisms. No bacteria or fungi were found on the agar surface of the culture medium and the roots and seed testa were likewise free of any organisms. In none of my experiments were seedlings obtained exhibiting the short blunt radicle characteristics of seedlings grown in the absence of the fungus as reported by Rayner.

Concurrently with my paper in the *New Phytologist* appeared a vigorous criticism of my experiments and conclusions by Rayner (6). Without subjecting my methods to a thorough test she rejects them as entirely inadequate for the purpose and specifically states that it is impossible to sterilise seed of *Calluna vulgaris* by means of calcium hypochlorite. Rayner emphasises the fact that cultures of the first series were contaminated as evidence that cultures of the second and third series were likewise not free from *Phoma radicis Cullunae*, particularly since the seedlings in those cultures developed an extended root system. She implies furthermore that I failed to observe infection of root cells because I sought infection similar to that found in typical mycorrhiza of *Calluna*. On the contrary I sought for the minutest evidence of infection since Rayner had made clear the almost evanescent character of the fungus-root association in seedlings.

The results that I obtained were so convincing to myself and entirely negative in respect to the necessity of the fungus for root development that no thought was given to a detailed presentation of the results. In view of the questions raised and the conclusions made by Rayner the problem was reinvestigated and the results of these new experiments are now presented.

MATERIALS AND METHODS

Source of seed. All of the seed were obtained in October, 1930, from the same plants from which seed were obtained for the earlier experiments. These plants are in the Nursery of the Department of Floriculture of the College of Agriculture at Cornell University. The plants have a spread of about 24 in. and are growing in a soil known as Volusia silt loam.

The plants are in excellent condition and flower regularly. These plants have been in the possession of the Department of Floriculture for more than ten years and have been in the present location for about seven years. The source of these plants is not known.

The seed were freed from the fruits by placing a mass of fruits between the hands and rubbing them in a rotary manner. The mass of material was then placed in a series of sieves and the seed and small particles of chaff obtained. The chaff was partially removed by a slow current of air. Finally the seed were hand picked so as to avoid the inclusion of any extraneous materials.

Sterilisation of seed (7). For this purpose the calcium hypochlorite method was used as described by Wilson. This method was developed

in our laboratory and has been used effectively with hundreds of different kinds of seed as well as for spores of ferns and mosses. In the preparation of the sterilising solution it is essential to use a calcium hypochlorite which has not deteriorated. Ten grams of the hypochlorite is added to 150 c.c. distilled water in a small flask. The mixture is then thoroughly shaken by hand for about 1 min. and then filtered. The filtrate is of course used. Wilson states that the filtrate should contain 2 per cent. chlorine, but from long experience with the method I have found that if the filtrate is faintly yellowish or amber in colour it will prove satisfactory. If the filtrate lacks this colour it is discarded and a new solution made from a fresh supply of calcium hypochlorite.

The seeds to be sterilised are placed in a small test-tube 4 cm. in length by 0·5 cm. in diameter. Enough of the hypochlorite solution is added to the tube so that the surface of the liquid reaches a mark one-third from the top. The mouth of the tube is closed by means of a finger and the tube is then shaken. Rayner (6) makes the point that air bubbles cling to the seed and that it would be impossible to wet them by means of hypochlorite. It is true that air bubbles do adhere to the seed, but by repeated agitation the seed are freed of bubbles. This may be acclerated by the use of a high-speed centrifuge. After about 20 min. practically every seed is bleached to a yellowish white colour and with slightly longer exposure most of the seeds become whitish in colour and are translucent. In the experiments here described the seeds were treated from 45 min. to 1 hour. The seed may be left in this solution for over an hour (the maximum time has not been determined) without any injury to the seed.

Some of the seed float to the surface and other seed settle to the bottom. By centrifuging most of the seed are thrown to the bottom of the tube. The floating seed are removed by decantation. In transferring the seed to the culture vessels use is made of an inoculating needle with No. 20 gauge platinum wire having a small loop at the end. With a little care one can pick up only a few seed or as many as fifty. The seed are not rinsed in distilled water previous to transferring them to the culture vessel. With the seed a thin film of the sterilising solution is of course transferred, but this is diluted by the culture medium and the hypochlorite soon disintegrates. No deleterious effect has been noted with any seed because of the transfer of the film of hypochlorite with the seed. Obviously the direct transfer without previously rinsing the seed in water reduces the chance of contamination.

Culture media. In my first experiments Rayner's solution A was used. In the experiments to be reported the media used were my solution B as used for orchid seedlings and a potato dextrose agar. In addition Rayner's solution with peptone and dextrose was used, but this medium will be described subsequently. The two culture media are as follows:

Solution B

$Ca(NO_3)_2\,6H_2O$ ...	I	grm.	$FePO_4$ ...	...	0·002 grm.
$(NH_4)_2SO_4$ ...	0·5	,,	Agar ...	...	17·50 ,,
KH_2PO_4	0·25	,,	Distilled water ...	I litre	
$MgSO_4\,7H_2O$ ...	0·25	,,			

In certain experiments I per cent. glucose was also added.

Potato dextrose agar

Sliced potato tubers (freed of peel)	...	200 grm.
Dextrose	10 ,,	
Agar-Bacto Difco brand	20 ,,	
Distilled water	I litre	

This mixture was autoclaved for 10 min. and then filtered through cotton: the reaction value of this medium is pH 6·0.

Culture tubes. The culture tubes used are 20·5 cm. in length with an internal diameter of 2·5 cm. Approximately 30 c.c. of the culture medium was placed in each tube. The tubes were autoclaved for 20 min. at 15 lb. pressure and then sloped to provide a large surface.

Culture conditions. All cultures were transferred immediately to a small compartment in the greenhouse. The temperature at night was maintained close to 60–65° F. The day temperatures ranged from 65 to 80° F. All tubes were maintained in a vertical position and shaded against the direct rays of the sun. Adequate light was provided and no unusual types of growth were noted.

Staining methods. In the experiment reported in my first paper, intact and crushed roots were examined microscopically for the fungus. In addition roots were fixed and imbedded in paraffin for microtome sectioning. These preparations were stained with Haidenheim's iron alum haematoxylon, a method found very satisfactory in my orchid work[1]. Rayner states ((6), p. 383) that "negative evidence yielded by examination of microtome sections of roots is quite valueless. The early stages of infection in cultures such as those

described show no hyphal complexes within the root cells, while the fineness of the mycelium, its causal distribution and liability to be washed away during manipulation render seed preparations useless." Assuming that the fungus had been in my cultures it seems remarkable that in the hundreds of slides prepared no fungus could be found nor was any noted in whole roots crushed under the cover glass. Since Rayner found the lactic acid phenol method with cotton blue stain of particular advantage, in the experiments here reported, intact roots were fixed, cleared and mounted in lactic acid phenol and stained with cotton blue dissolved in the lactic acid phenol. The lactic acid phenol was made as follows: 100 grm. phenol, 100 grm. lactic acid, 100 grm. glycerol and 100 c.c. distilled water. To one-half of this solution was added 1 grm. cotton blue.

In preparing the roots for examination they were first placed in the lactic acid phenol in a watch glass. This was heated momentarily by means of several lighted matches. The lactic acid phenol mixture was then replaced by the cotton blue solution and again heated for a few seconds. The material was again transferred to the lactic acid phenol momentarily and then mounted in the lactic acid phenol. Preliminary tests with various fungi, roots obtained from *Calluna vulgaris* growing in pots in the greenhouse and orchid roots proved the satisfactory character of the method for staining fungal hyphae.

EXPERIMENTAL

Exp. 5. On December 8th, 1930, sixteen cultures were prepared with solution B adjusted to *p*H value of 4·9. A like series was prepared with solution B plus 2 per cent. cane sugar adjusted to a *p*H value of 4·9. Ten to fifteen seed were placed in each tube and the tubes immediately transferred to the greenhouse. After 6 days some of the seed had germinated with the radicles a few millimetres in length. These were noted in tubes with sugar as well as in tubes without sugar. At this time no micro-organisms were apparent on the seed coats or on the agar slope. About fifteen seedlings were removed from each series and stained by the lactic acid phenol cotton blue method. The seed coats were also treated in the same way and mounted for microscopic observation. In no case could any fungal hyphae be observed nor was there any evidence of any micro-organisms. Magnifications up to 950 were used.

From December 14th to 25th seedlings were removed from each series at intervals of 2 or 3 days, stained and mounted. No fungus or

any other organism could be found, and the culture media remained free of any contaminating organism.

These cultures were continued until March 17th, 1931 (Pl. VI, fig. 1). At this time the seedlings were well developed with long roots. The agar surfaces of all cultures were free of any micro-organisms. From the cultures containing solution B without sugar one seedling was removed from each of five tubes. The seedlings were uniform in appearance and size and had an average stem length of 22 mm. Each seedling was quickly transferred to a large culture tube 200 × 20 mm. containing potato dextrose agar. Precautions were observed to prevent the entrance of micro-organisms from the atmosphere. These tubes were then placed in an incubator and maintained in darkness at a temperature of 26° C. After 1 month in the incubator the leaves began to turn brown, an exudate was present on the leaves and after 6 weeks the plants appeared to be dead. No fungus or any other micro-organisms developed on the plants and the agar surfaces were entirely free of any micro-organisms. These tubes were kept in the incubator for 8 months and remained free of any microbial growth during this period of time.

Exp. 6. Potato dextrose agar is a favourable medium for the growth of many fungi. It appeared probable that if any fungus were present in the seed coat or present on the surface of the embryo it would grow on the potato dextrose agar. For comparison my solution B with a reaction of 4·9 was used and the same solution + 2 per cent. cane sugar. Five cultures with each culture medium were provided with from five to eight seeds in each tube. Most of the seed germinated within 7 days. Thereafter growth was entirely normal except in the potato agar cultures. In these tubes the radicles were short and the tips soon became brownish in colour. These seedlings continued to survive for some time, but by June 8th, 1931, practically all of these seedlings were dead. In none of the cultures was any growth of micro-organisms noted. Seedlings and seed coats were removed from two tubes of each series. These were stained by the lactic acid phenol cotton blue method and examined carefully for fungus hyphae. None were found. Stem and root measurements were made on each plant. The results are given in Table I.

The remaining tubes were maintained until November 17th, 1931. By this time the seedlings with sugar had an average height of approximately 25 mm. and an average root length of 46 mm. Without sugar the averages were 21 and 50 mm. respectively for stems and roots. With sugar provided, a greater growth of roots was observed

than without sugar, for while the average length was about the same those seedlings provided with sugar showed a much more branched root system. The roots from these plants were stained in the usual manner and observed microscopically. No fungus or any other organism was noted. The cultures remained free of any micro-organisms.

<div align="center">TABLE I</div>

<div align="center">

Growth of seedlings on various culture media.
March 17th to April 20th, 1931

Measurements in millimetres

</div>

Solution B		Solution B + 2 % cane sugar		Potato dextrose agar	
Height of stem	Length of roots	Height of stem	Length of roots	Height of stem	Length of roots
6	4	5	7	6	1
8	6	6	5	6	1
3	2	4	4	4	0·6
6	5	4	5	4	0·5
2	1·5	4	6	5	1
3	2	6	6	6	0·4
1	1	4	3	5	4·0
3	3	3	1	6	0·5
4	6	6	2	5	1
6	8	4	7	5	1
5	6	6	5	3	0·5
3	5	6	·7	6	0·6
—	—	4	5	5	1·0
—	—	7	6	4	0·5

Exp. 7. The seedlings produced on potato dextrose agar were so surprising in character and so similar to the asymbiotic seedlings described by Rayner [3, 4, 5] that it seemed desirable to repeat the experiment. Seven lots of seed were treated with the hypochlorite for periods of 1, 5, 10, 15, 20, 30 and 40 min. Seed from each lot were transferred to tubes of solution B + 2 per cent. glucose and to potato dextrose agar. The cultures were then placed in the greenhouse. The experiment was begun on May 22nd and concluded on June 23rd, 1931. By this time nearly all of the seedlings on the potato dextrose agar were much retarded, with short radicles and the tips of the radicles yellowish or brownish in colour. These seedlings made no further development after June 23rd and practically all were dead by August 1st. The root measurements of these cultures are given in Table II and representative plants are shown in Pl. VI, figs. 2 and 3.

The results for this experiment and those of Exp. 6 are significant. On potato dextrose agar the roots do not develop. No fungus was present either in the roots or on the culture medium. The seedlings

TABLE II

*Influence of time of treatment of seed with hypochlorite and comparison
of solution B + 2 per cent. glucose and potato dextrose media.
Experiments begun May 22nd. Concluded June 23rd, 1931*

Time of treatment with hypochlorite min.	Solution B + 2 % glucose mm.	Potato dextrose agar mm.
1	—*	—*
5	8·0 ⎫	
10	8·6 ⎪	
15	5·2 ⎬	Less than 1
20	8·2 ⎪	
30	8·0 ⎪	
40	7·7 ⎭	

* Contaminated with *Chaetonium* sp.

resembled those described by Rayner produced in the absence of the
fungus. Yet seed taken from the same lots were placed at practically
the same time in tubes containing solution B + 2 per cent. glucose.
These seedlings developed in a normal manner with well-developed
roots. No fungus was found in the roots of these plants nor in the
culture medium. Seedlings on solution B without sugar develop in a
manner like those on solution B plus sugar.

Exp. 8. The results with potato dextrose agar indicate that either
potato dextrose agar is toxic or that it is lacking in some essential
nutrient, the deficiency of which prevents root growth. The experi-
ments suggest furthermore that the peptone dextrose medium used by
Rayner may have been toxic to the *Calluna* seedlings. Rayner states
((6), p. 382) "In the case of my original cultures sterilised seeds were
sown upon a medium rich in sugar and nitrogenous material." From
this medium seedlings were transferred to the culture vessels con-
taining the requisite nutrient salts. In the main portion of Rayner's
original paper (3) no mention is made of the exact composition of the
culture medium on which the seed were first placed except the state-
ment that the agar medium contained peptone, dextrose and nutrient
salts. In the appendix to the paper (p. 129) is given the following
culture medium: Rayner's solution A + 0·1 per cent. dextrose + 0·1
per cent. Witte's peptone + 0·12 per cent. agar. Obviously this
formula is not correct, for with 0·1 per cent. agar the culture medium
would remain liquid. It is probable that 1·2 per cent. agar was used.
Since the wrong concentration of agar was reported it is highly
probable that the same error was made relative to the concentration

of dextrose and peptone. The concentrations of the substances probably should have read 1·0 per cent. dextrose and 1·0 per cent. peptone. These concentrations are what one would ordinarily employ in cultures for fungi and bacteria, and since Rayner refers to the richness of sugar and peptone the conclusion is permissible that 1 per cent. dextrose and 1 per cent. peptone were used.

To determine if such a culture medium has the same effect as the potato dextrose agar an experiment was made using Rayner's solution A plus peptone and dextrose in two different amounts. For comparison my solution B + 2 per cent. dextrose was also used.

Rayner's solution A was prepared as follows:

Potassium nitrate	...	...	...	1 grm.
Magnesium sulphate		...	...	0·4 ,,
Calcium sulphate	...	...	...	0·5 ,,
Monobasic calcium phosphate		...	0·5 ,,
Sodium chloride	...	...	...	0·5 ,,
Ferric chloride	...	...	...	5 mg.
Water ...	...	...	...	...	2000 c.c.

The three culture series were prepared as follows:

(1) Rayner's solution A + 1 per cent. dextrose + 1 per cent. peptone + 1·2 per cent. agar.

(2) Rayner's solution A + 0·1 per cent. dextrose + 0·1 per cent. peptone + 1·2 per cent. agar.

(3) Knudson's solution B + 2 per cent. dextrose + 1·75 per cent. agar.

Seed of *Calluna* were treated for 50 min. with the hypochlorite

Table III

Effect of various culture media on seedling development. Experiment begun November 18th, 1931. Concluded January 4th, 1932

Culture medium	pH	Culture No.	No. seeds in culture	No. seed-lings	Av. length tops mm.	Av. length roots mm.
Solution B + 2 °/₀ glucose	4·9	C 1	10	8	4·7	5·1
Solution B + 2 °/₀ glucose	4·9	C 2	10	10	5·4	4·9
Rayner's solution A + 1 °/₀ glucose + 1 °/₀ peptone	6·1	C 4	16	7	4·0 ⎫	Less than 1/2
		C 5	26	2	4·0 ⎪	
		C 6	35	13	4·4 ⎬	
		C 7	13	3	3·3 ⎭	
Rayner's solution A + 0·1 °/₀ glucose + 0·1 °/₀ peptone	6·0	C 8	26	18	4·8	7·0
		C 9	46	21	4·9	6·9
		C 10	27	25	5·0	7·2

and transferred to the tubes. The experiment was begun on November 18th, 1931 and concluded on January 4th, 1932. The results on growth are given in Table III and representative plants are shown in Pl. VII, figs. 4, 5 and 6.

No fungi or other micro-organisms were to be found on the culture medium or the root and seed coats and the roots were free of infection. Of great importance is the failure of roots to develop in the cultures with 1 per cent. peptone and 1 per cent. dextrose. To Rayner this would indicate that the fungus was not present yet cultures prepared at the same time with Rayner's solution A + 0·1 per cent. peptone and 0·1 per cent. dextrose with solution B + 2 per cent. glucose developed normally with good root systems. The same lot of seed was used for all cultures. The significant fact is that peptone is toxic. In addition to the injury to roots there was a marked reduction in the percentage of germination.

DISCUSSION

It is Rayner's contention that root development and consequently seedling development of *Calluna* is dependent on infection of the seedling by the appropriate fungus. In her various papers, figures are given to show the stubby character of roots in the absence of the fungus. In my first paper I reported successful seedling development without the fungus, and in the experiments here reported no fungus was found either in the roots, on the seed coats or on the agar surface, and still well developed roots were produced by the seedlings.

Rayner questioned the adequacy of the hypochlorite method for sterilising the seed and stated also that the nutrient solution used in my first experiments(2) would not permit the development of any micro-organisms. On an agar medium with only nutrient salts many fungi do develop (of course in a weak manner), but such growth is readily visible. With inadequate sterilisation growth of micro-organisms may be noted on the seed coat or about sloughed off root cap cells as well as on the surface of the root. That calcium hypochlorite properly used is effective in ridding seed of micro-organisms has been clearly demonstrated by many experiments in our laboratory.

That calcium hypochlorite is effective for sterilising the seed is evident from Exp. 6 in which one series contained potato dextrose agar. This is an exceptionally good medium for the growth of many fungi and bacteria. In Exp. 7 potato dextrose agar was again used, and a treatment of the seed for only 5 min. freed the seed of micro-organisms so that pure cultures were obtained. Again in Exp. 8 a

culture medium was used which Rayner found favourable for the fungus *Phoma radicis Callunae*. Again no growth of any micro-organisms was found either on the seed coats, surface of the agar, on the roots or within the roots. Furthermore, transfers of well-developed seedlings were made to tubes containing potato dextrose agar. These tubes were incubated at 26° C. in the dark. No micro-organisms appeared even after many months' incubation. It seems incredible to me that with such favourable culture media the fungus would never develop on the culture media if it were present on or within the seed.

As stated previously Rayner after treating the seed with mercuric chloride and rinsing them repeatedly with distilled water transferred the seed to agar plates. The medium contained nutrient salts, peptone and glucose in order to facilitate the growth of micro-organisms. "By this means a number of plates of seedlings were obtained which remained entirely free from infection by micro-organisms" (Rayner (1918), p. 105). In addition some tests for sterility were made by transferring some of the seedlings to other media and the results were in agreement with the observation on the peptone dextrose medium. In other words if no micro-organisms were apparent on the seed or seedlings on the surface of the medium it was assumed that the mercuric chloride had effectively sterilised the seed. I submit that similar freedom of micro-organisms on nutrient solutions plus sugar, on potato dextrose agar, and on Rayner's solution plus peptone and dextrose is just as good evidence as Rayner's for the purity of the cultures.

These cultures were examined not only with a hand lens but by means of a dissecting binocular. In addition surface portions of the agar medium were examined under magnifications of 950, and observations were made of stained and unstained roots at similar magnifications. When it is stated therefore that the cultures were free of micro-organisms these various criteria were involved.

In my earlier paper I suggested an explanation for the stubby character of the roots of asymbiotic seedlings as reported by Rayner. The appearance of these stubby roots is characteristic of a toxic condition, and the more probable cause of the toxicity I assumed to be the mercuric chloride since a concentration of 1 per cent. was used.

In my own Exps. 6, 7 and 8 seedlings similar to the asymbiotic seedlings of Rayner were obtained on potato dextrose agar and on Rayner's solution A plus 1 per cent. peptone and 1 per cent. dextrose. This latter solution was used by Rayner for the initial germination

of the seed, and from plates containing this culture medium seedlings were transferred after about 21 days to the culture tubes. That the peptone is toxic is revealed by the fact that seed adhering to the wall of the tube out of contact with the peptone would develop a normally elongated root of healthy appearance. That other substances may inhibit root growth is shown by the result on potato dextrose agar. Some preliminary experiments with my solution B with a pH value of 6·6 gave indications of stubby roots though not so pronounced as on the peptone or potato dextrose agar.

I have not been able to obtain the fungus from *Calluna*. Following Rayner's (4) procedure I obtained only *Alternaria* and *Chaetonium*. It has been impossible therefore to determine the action of the fungus on seedlings grown initially on potato dextrose agar and on Rayner's solution A plus 1 per cent. peptone and 1 per cent. dextrose. It is conceivable that when seedlings are transferred from the peptone plates some peptone adheres to the roots and basal portion of the stem. Unless this peptone is removed a continued toxic condition prevails. Peptone is readily utilised by many fungi and it is possible that the favourable effect reported by Rayner when the fungus is supplied may be due to the destruction of the peptone.

Rayner's conclusions with respect to the necessity of the fungus were made on the basis of experiments using a medium containing peptone. With a favourable culture medium with or without soluble organic matter seedlings in my experiments develop with healthy normal roots and without the aid of the fungus. Seedling development of *Calluna vulgaris* therefore does not require the aid of *Phoma radicis Callunae*.

SUMMARY

Seedlings of *Calluna vulgaris* develop roots on various culture media with or without sugar and without the intervention of any fungus.

By germinating seedlings on potato dextrose agar, solution B plus sugar and on Rayner's peptone dextrose agar, adequate proof of the purity of the cultures was obtained.

Well-developed seedlings were transferred to tubes containing potato dextrose agar. Such tubes were incubated for 8 months. No micro-organisms appeared on the seedlings or culture medium.

No fungus could be found on the seed coats, on the roots, or within the roots of any seedlings examined microscopically before or after appropriate staining.

Fig. 2

Fig. 3

Fig. 1

KNUDSON—NON-SYMBIOTIC DEVELOPMENT OF
CALLUNA VULGARIS
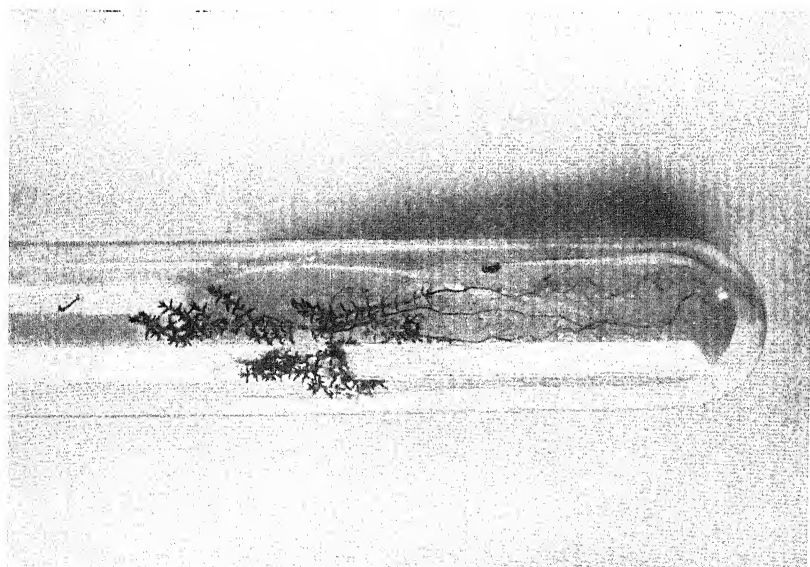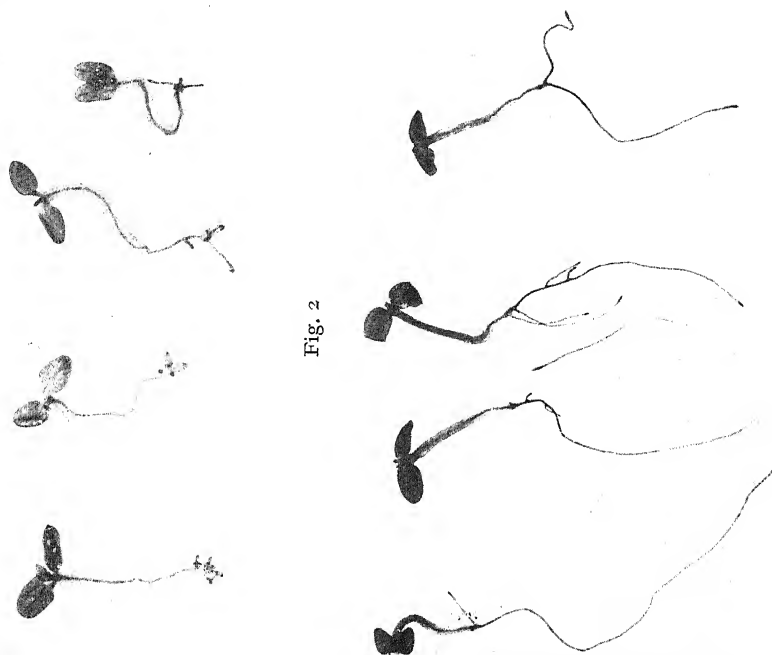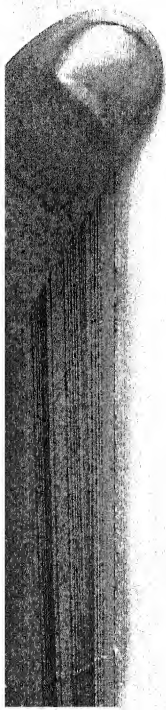
Fig. 4
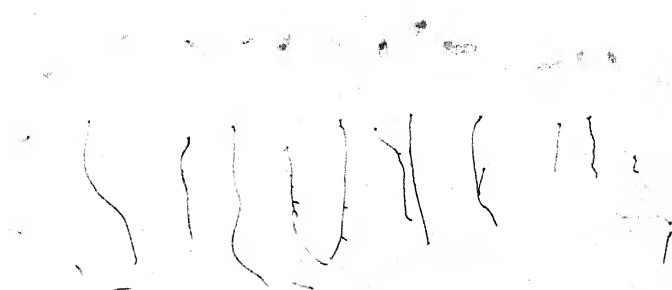
Fig. 5

Fig. 6

KNUDSON—NON-SYMBIOTIC DEVELOPMENT OF
CALLUNA VULGARIS

Potato dextrose agar as well as the peptone dextrose agar as used by Rayner proved to be toxic to the roots of *Calluna vulgaris*.

It is concluded that the stubby roots noted by Rayner are due to a toxic action of the peptone dextrose medium and that *Calluna vulgaris* does not require the fungus for proper root development.

These investigations substantially support my conclusions of an earlier paper.

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EXPLANATION OF PLATES

PLATE VI

Fig. 1. Seedling *Calluna vulgaris* on solution B. Age 100 days.
Fig. 2. Seedlings grown on potato dextrose agar. Note stubby roots.
Fig. 3. Seedlings grown on solution B + 2 per cent. glucose.

PLATE VII

Fig. 4. Seedlings on Rayner's solution A + 1 per cent. peptone and 1 per cent. glucose. Note stubby roots.
Fig. 5. Seedlings on Rayner's solution A + 0·1 per cent. peptone + 0·1 per cent. glucose.
Fig. 6. Seedlings on solution B + 2 per cent. glucose.

THE RÔLE OF ORGANIC ACIDS IN PLANT METABOLISM

PART II[1]

By T. A. BENNET-CLARK

(With 8 figures in the text)

CONTENTS

SUCCULENT PLANT METABOLISM; INTRODUCTION

IT has been known since the first decade of the nineteenth century that some members of the Crassulaceae possess the peculiarity that they produce large quantities of an acid, which is now known to be malic acid, when they are placed in darkness. Since the quantities of CO_2 which they produce are rather remarkably small in comparison with the quantity of malic acid produced, the view became established that this acid production was an abnormal type of respiration, in which a product is formed less highly oxidised than the carbon dioxide which is the normal end-product of respiration. On this simple view

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the respiratory and photosynthetic processes of the Crassulaceae can be summarised in the equation

$$2C_6H_{12}O_6 + 3O_2 \underset{\text{Photosynthesis}}{\overset{\text{Respiration}}{\rightleftharpoons}} 3C_4H_6O_5 + 3H_2O.$$
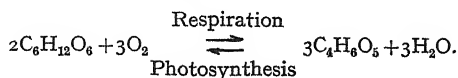
A number of views have been put forward as to the mechanism of this peculiar Crassulacean metabolism, which is, however, found in many species and genera of plants belonging to the most diverse Natural Orders. This metabolic peculiarity is found both among succulent plants (almost all of which exhibit it) and among non-succulent plants.

Broadly speaking two types of hypothesis of the mechanism of the process are possible. Some investigators have held that the malic acid is formed by the gradual oxidation of sugar, and that eventually the malic acid is oxidised step by step to oxalic acid and finally to carbon dioxide and water. This view of the gradual step by step oxidation of the sugar in respiration is clearly quite distinct from the course of sugar breakdown which is associated with the zymase complex of enzymes. This latter mode of glycolysis involves the formation of 3-C compounds, such as methyl-glyoxal, from hexoses. This zymasic sugar breakdown is found in tissues of very widely divergent affinities, the classical examples being yeast and muscle; zymase is also widely distributed in the plant kingdom. The view is therefore widely held that the zymase complex of enzymes is responsible for sugar break-down during respiration (Kostytchev[27]) in all living tissues, and, therefore, that the 4-C acids like malic acid must arise otherwise than by the direct oxidation of hexoses.

In conformity with this point of view, Kostytchev supposes that the plant acids arise from proteins and not from carbohydrate sources, and this view is upheld by Ruhland and Wetzel[42, 43, 44]. Since the evidence in the case of the succulent plants is in complete contradiction to this view, as pointed out in the last section, Ruhland and Wetzel[45] admit that in this particular case the malic acid probably arises from 3-C or 2-C compounds formed from sugar breakdown.

It must be admitted that if the dogma is reasonable that the course of glycolysis is essentially similar in all plants, and this dogma underlies the modern theories of acid metabolism, then it would be equally reasonable to hold that a corresponding uniformity in the mode of synthesis of the plant acids exists.

It is, however, not yet quite clear that glycolysis does necessarily involve the operation of the zymase system. Recent work (Lunds-

gaard(33)) shows that some part of the zymase complex is paralysed by solutions of iodacetates. In spite of the cessation of alcohol and CO_2 production by the zymasic breakdown of sugar in yeast, it was, however, found that yeast still continued to produce CO_2. So the possibility remains that sugar breakdown occurs by other processes than the "Neuberg reactions" which the zymase complex induces.

We will therefore deal with three groups of hypotheses.

(A) Those which suppose that the respiration and acid formation do not involve the "Neuberg reactions," but hold that a step by step oxidation of hexoses to organic acids occurs.

(B) Those which suppose that sugar breakdown in respiration involves the "Neuberg reactions," and which hold that acid formation results from secondary or side reactions imposed on the main downgrade drift of reactions.

(C) Those which suppose that sugar breakdown involves the "Neuberg reactions," and which suppose that the organic acids are not produced from sugar during respiration, but that they are produced either from proteins or are produced as by-products of protein synthesis. In view of the clear evidence that the malic acid of succulent plants does not originate in protein metabolism, this type of theory will not be dealt with in the present section of this review.

A. HYPOTHESES WHICH DO NOT INVOLVE THE NEUBERG REACTIONS

The hypothesis, which has dominated most accounts of the metabolism of succulent plants from the time of Pfeffer to the present day, supposes that the accumulation of organic acids during darkness is connected with the shortage of oxygen in the internal atmosphere of the succulent plant. The work of Warburg(53) and Aubert(4) was held to support this view. These authors point out that it is greatly to the advantage of xerophytes, such as the succulent plants, in which gaseous exchanges are hindered, that carbon dioxide should not be lost from the tissues during respiration.

At first the purely teleological explanation was advanced that it was advantageous when a large bulk of tissue communicated with the external air through a relatively small stomatal area, as in the succulent plants, that the tissue should not lose CO_2 during respiration, but that its carbon resources should be conserved by the oxidation of sugars to products, such as malic acid, which are retained in the tissues. Aubert believed that his results showed that the extent of acid accumulation was proportional to the degree of succulence.

Exceptions to this rule are many, and even the classical example of *Bryophyllum* hardly conforms to Aubert's rule as its acid metabolism is "active," and its leaves are broad and rather thin: in any case it is not possible to put sufficiently precise quantitative interpretation to Aubert's rule.

From this teleological view of the connection between acid accumulation and degree of succulence, there developed the view that the two phenomena were causally related, which still forms the basis of most accounts of the subject. It has been accepted as recently as 1919 in original work on succulent plant metabolism (cf. Spoehr[49]). It is held that the oxygen tension in the internal atmosphere of succulent plants is abnormally low and that this abnormal oxygen tension causes the sugars to be incompletely oxidised, i.e. instead of being oxidised to CO_2 and water, they are oxidised to malic acid.

The earliest investigators produced evidence which is at complete variance with the expectations based on this hypothesis. Warburg in 1886, and Astruč in 1903, for example, showed that greater accumulations of malic acid occurred when the oxygen tension was artificially increased and conversely smaller accumulations of acid formed when the oxygen tensions were lowered. Richards' results on *Opuntia* differ from this, it must be admitted. But Richards and all other investigators agree that a decrease in temperature brings about a *rise* in acidity; this change in temperature clearly must bring about a *rise* in the oxygen tension of the tissues.

No experimental evidence exists as to the composition of the internal atmosphere in succulents. Aubert made some analyses but he obtained his gas samples by introducing leaves into a Torricellian vacuum, and therefore the intercellular gas was contaminated with CO_2 which must have come out of solution owing to the much reduced pressure.

In spite of this lack of evidence Richards[40] summarises the position in the words "it would seem as if the normal respiration of the cacti were of the nature of what is commonly called intramolecular, except that the process is not carried far enough to yield alcohol, but stops at malic acid." The term intramolecular respiration is supposed to indicate that the oxygen used to oxidise certain carbon compounds to CO_2 is obtained by the reduction of other carbon compounds: the result of this is that products poorer in oxygen than carbohydrates such as alcohol or acetone accumulate.

If the "intramolecular respiration is not carried far enough to yield alcohol" one would expect a product intermediate in com-

position between the carbohydrates and alcohol (i.e. between $(C_2H_4O_2)_x$ and $(C_2H_6O)_y$) and not a product like malic acid which is more highly oxidised than the carbohydrates themselves.

More recently Evans[18] has pointed out that the work of Schroeder[47] and Romell[41] is not favourable to the view that the oxygen tension in the intercellular spaces of succulents is abnormally low, for these workers find that the resistance to the inward diffusion of oxygen opposed by the stomata and the diffusion path through the intercellular spaces is much less than that offered by the water-air interface of the cell.

One may summarise the position by stating that: (a) There is no evidence of oxygen shortage in succulent plant tissues, but rather the reverse. (b) Even if there were a shortage of oxygen, other than an almost complete absence of oxygen, there is no reason to suppose that this would modify the nature of the products formed during respiration; such modification is not found in other plants. A change in rate of respiration would be expected, not a change in its mechanism (cf. Blackman[10]). (c) Even if one admits the possibility of "partial anaerobiosis," the expected products would not be more highly oxidised than the sugars. (d) Experimental results of all workers, except Richards, on the effects of artificial gas mixtures on the succulents are definitely opposed to this partial anaerobiosis theory. The experimentally observed effects of temperature change on the acid content are also opposed to this view.

Mayer first put forward the view that the malic acid formed in the succulents was formed in respiration by the partial oxidation of sugars. Kraus[28, 29], de Vries[16, 17], and Purjewitsch[39] extended this view. de Vries first emphasised that formation and decomposition of the malic acid were both taking place continuously, both when the tissues were exposed to light and to continued darkness. High temperatures favoured the decomposition process, and so also did illumination of the tissues.

Purjewitsch found that in the Crassulaceae (*Aeonium*) the acid content reached its maximum value about 8 hours after the leaves had been placed in darkness. In other cases (*Robinia*, for example) the maximum value of the acidity was found after a longer period in darkness, 24 hours. This was related by Purjewitsch to the supposedly greater stability of the citric acid found in *Robinia*.

Nathansohn[37] accepted these views which may be summarised by writing the succession of reactions involved in the respiration of succulent plants thus:

$$\text{Sugars} \rightarrow \text{malic acid} \rightarrow CO_2 + H_2O.$$
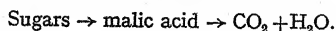
de Vries attempted to give some explanation of the fact that malic acid alone of all the various intermediate products accumulated in high concentration, but the explanation is very inadequate. He suggested that the accumulation was due to the stimulus of light. The suggestion that acid accumulation is due to oxygen scarcity has been dealt with.

Nathansohn ((37), p. 318) and Pfeffer(38) both point out that the evidence is against the view that oxygen shortage has this effect. Nathansohn's account of the matter is particularly clear, and he introduces the three lines of evidence which were dealt with in the preceding section. He gives, however, no explanation of the accumulation of malic acid; both he and Pfeffer simply state that it is due to the specific nature of the plants.

The present writer(7) apparently first attempted to define a specific property of the plants which would cause this accumulation. If a succession of chemical changes takes place of the type

$$\text{Sugar} \underset{k_1}{\rightarrow} \text{malic acid} \underset{k_2}{\rightarrow} X \underset{k_3}{\rightarrow} CO_2,$$
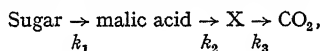
the concentrations of the various reactants at various times after the start of the reaction (only sugar is supposed to be present at the start) can be calculated if the magnitudes of the velocity constants (k_1, k_2, k_3) are known (Rutherford(46), pp. 420–3). Without going into exact quantitative details (since the values of the constants in this case are unknown) it can be seen readily that if $k_1 < k_2 < k_3$, then the concentrations of malic acid and X will be small during the whole course of the conversion of sugar to CO_2.

But if $k_1 > k_2 < k_3$ the concentration of malic acid will rise to a maximum value and will finally fall off from this maximum value roughly logarithmically. At the maximum acidity the rate of acid formation and decomposition are equal and it follows that

$$k_1 \text{ [sugar]} = k_2 \text{ [malic acid]},$$

so that since $k_2 < k_1$, the concentration of the malic acid must have risen above that of the sugar.

The exact relationships are shown in Fig. 1 for a system in which the velocity constants are known: the breakdown of radium A

$$\text{Ra A} \rightarrow \text{Ra B} \rightarrow \text{Ra C} \rightarrow \text{Ra D}.$$

The half lives of each of these elements are 3, 28·6, and 19·5 min. respectively, so the velocity constants correspond to the series $k_1 > k_2 < k_3$ mentioned above. The ordinates of the curves represent the relative numbers of molecules of A, B, C, and D at any time, taking the initial amount of A as 100.

Curve A may be taken to indicate the change of sugar concentration in the system just described with time, curve B indicates the change in malic acid concentration with time when, as we have assumed, $k_1 > k_2$. This latter curve should be compared with the experimentally observed results of change of acidity of succulent plants which are collected in Fig. 2.
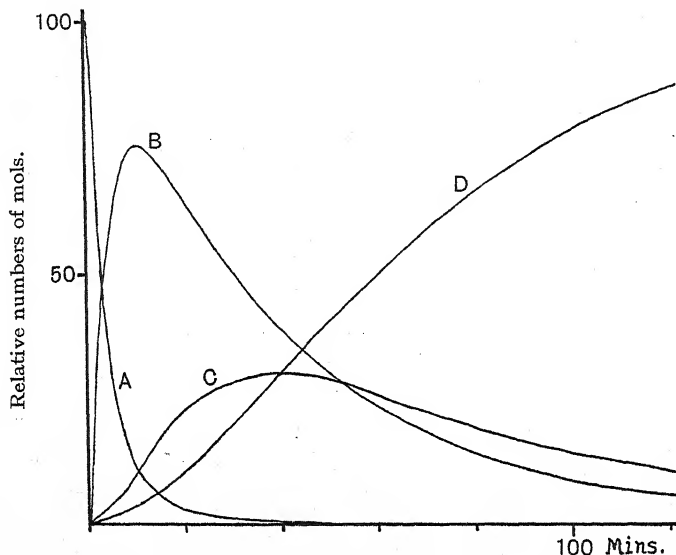
Fig. 1. Relative quantities of the reactants, at times indicated as abscissae, in reaction $A \to B \to C \to D$ where $k_1 > k_2 < k_3$. For further discussion see text.

The curves given in Fig. 2 illustrate the change in acidity of cut leaves placed in continuous darkness. At the commencement of the period of darkness much sugar is present (due to the previous photosynthesis) but little acid. The starvation in darkness causes at first a rise in acidity which is followed by an approximately logarithmic decrease in acidity to a low value. The type of curve closely resembles curve B in Fig. 1.

One therefore considered that the cause of acid accumulation in succulents lay in the relative activities of the enzyme systems which determine the values of k_1 and k_2. The lack of acid accumulation in plants which do not contain large quantities of malic acid was provisionally supposed to be due to different relative efficiencies of these two enzyme systems such that $k_1 < k_2$ in plants which contain little acid. It is possible that this is exactly what Pfeffer and Nathansohn

meant when they stated that acid accumulation was due to the specific nature of the plant, but precise formulation of the process as a series of successive reactions was not attempted by them.
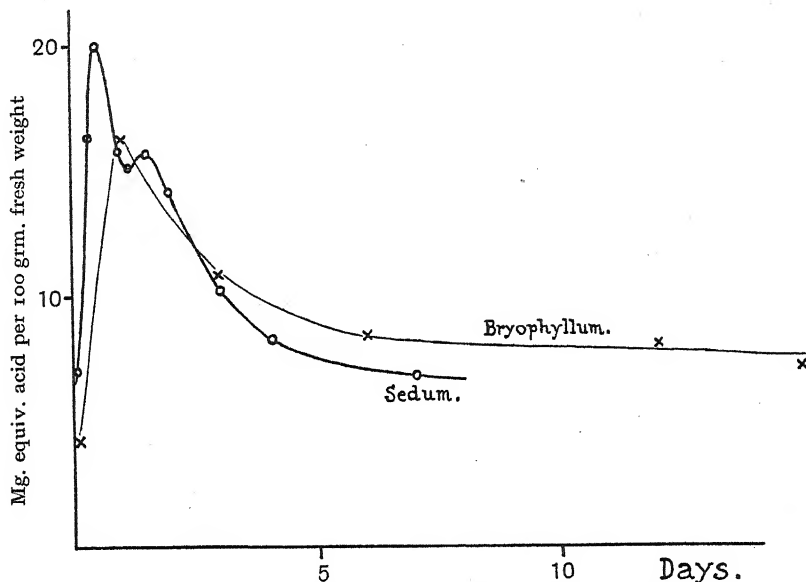
Fig. 2. Titratable acidities of leaves of *Bryophyllum* (Gustafson (20)) –×—×– and excised leaves of *Sedum praealtum* (Bennet-Clark) –o—o– kept in continued darkness at constant temperature. Each point in latter curve shown thus –o– is the average of 4 analyses; the *Sedum* leaves were darkened at 6 p.m. on June 2nd.

This view of the cause of acid accumulation does not involve one necessarily in the assumption that CO_2 is formed from malic acid or a breakdown product of malic acid. Acid accumulation in the manner indicated would occur in the system

$$\text{Sugar} \rightarrow A \rightarrow \text{malic acid} \rightarrow \text{polysaccharide,}$$
$$\downarrow$$
$$CO_2$$
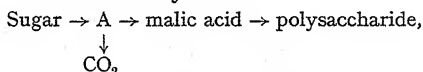
provided that the velocity constant of formation of acid exceeded that of its decomposition.

B. Hypotheses based on the Neuberg reactions

(a) An outline of the views of the Leipzig school

Two hypotheses come within this section: that of Ruhland and Wetzel (45) and Wolf (55), and the present writer's (7, 8). The Leipzig theory is based largely on recent work which has been done on the

formation of the 4-C acids (succinic, fumaric, and malic acids) in muscle.

Carbohydrate breakdown in muscle respiration is supposed to follow the "Neuberg schema," and, accordingly, the view was first put forward that succinic acid was formed from acetic acid by the Thunberg reaction, thus:

$$
\begin{array}{l}
CH_3.COOH \\
+ \qquad\qquad -2H = \quad \begin{array}{l} CH_2.COOH \\ | \\ CH_2.COOH \end{array} \\
CH_3.COOH
\end{array}
$$
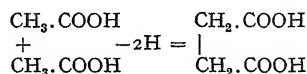
so the whole sequence of reactions by which succinic acid was supposed to be formed is as follows:

Sugar → methyl-glyoxal → pyruvic acid → acetaldehyde → acetic acid → succinic acid.

Toeniessen and Brinkman (52), however, showed that acetic acid was not converted by muscle into succinic acid, but that succinic acid is formed when pyruvic acid is perfused through muscle tissue. The pyruvic acid disappears and succinic and formic acids are found in the perfusate. They suggest therefore that the conversion of pyruvic acid to succinic acid occurs by the following stages:

$$
\begin{array}{l}
CH_3.CO.COOH \\
+ \qquad\qquad -2H = \quad \begin{array}{l} CH_2.CO.COOH \\ | \\ CH_2.CO.COOH \end{array} \\
CH_3.CO.COOH
\end{array}
$$

pyruvic acid diketoadipic acid

$$
\begin{array}{l}
CH_2.CO.COOH \\
| \qquad\qquad +2H_2O = \quad \begin{array}{ll} CH_2.COOH & H.COOH \\ | & + \\ CH_2.COOH & H.COOH \end{array} \\
CH_2.CO.COOH
\end{array}
$$

diketoadipic acid succinic acid formic acid
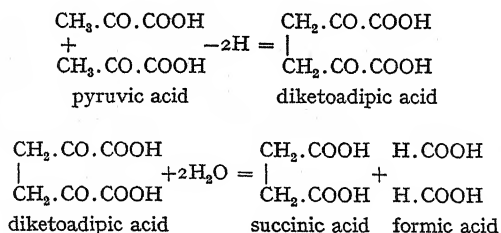
Diketoadipic acid is supposed to be formed from pyruvic acid by a modification of the Thunberg reaction as indicated, and it is then hydrolysed to give succinic and two molecules of formic acid. It may be mentioned that this diketoadipic acid has not been detected, nor is it known at present whether it is rapidly converted to succinic and formic acids. Rapid hydrolysis would account for the fact that it has not been observed to accumulate in sufficient concentration to permit of its detection.

The Leipzig school consider it probable that the respiration of succulent plants involves splitting of sugars by zymase in the same way that is observed in most other tissues, and that the fate of the

pyruvic acid is different from what is regarded as its usual fate in the plant kingdom. The process is thought to be:

Hexose
↓
Methyl-glyoxal
↓
Pyruvic acid → diketoadipic acid → succinic acid
↓ ↓
Aldehyde +CO_2 malic acid
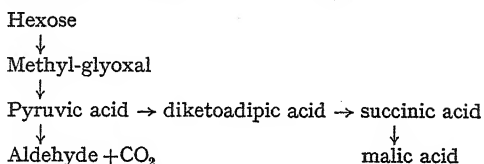
The normal fate of pyruvic acid is thought to be its decomposition to aldehyde and CO_2 under the influence of the enzyme carboxylase. The Leipzig school hold that the activity of the carboxylase is lessened in the succulent plants by an inhibitor, with the result that pyruvic acid is diverted into the side reaction indicated above.

The chief reason for holding such a view is the quite reasonable belief that the Neuberg reactions (hexose → methyl-glyoxal → pyruvic acid → aldehyde + CO_2) account for the sugar breakdown.

Now it is well known that many fungi, notably *Mucor*, *Aspergillus* and *Penicillium* species, are capable of producing 4-C acids and also citric acid when supplied only with alcohol, aldehyde, or acetic acid (Butkewitch and Fedoroff[13], and Chrząszcz and Tiukow[14]). This conversion of 2-C into 4-C compounds is naturally supposed to be brought about by an oxidation of the type of the Thunberg reaction (see p. 136).

Consequently the scheme of reactions given below seems very plausible:

Hexose → 2 methyl-glyoxal → 2 pyruvic acid → 2 aldehyde +2CO_2
↓
2 acetic acid
↓
Succinic acid
↓
Malic acid
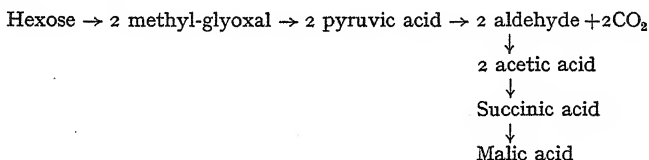
clearly since two molecules of acetic acid are converted into one of malic acid, the formation of each molecule of malic acid will be associated with the formation of two molecules of CO_2.

Wolf[55] points out that this necessity rules out the possibility of occurrence of these reactions in succulents, since the quantity of CO_2 formed during malic acid formation is not nearly adequate to account for its formation from pyruvic acid in the manner indicated above. This is made clear by the results given in Table I, some taken from Wolf, and some from the writer's results.

The Toeniessen-Brinkman schema seems consequently a more likely mode of origin of the 4-C acids from the 3-C products of glyco-

TABLE I

*Relative quantities of malic acid and CO_2 formed
on darkening succulent plant tissues*

Material and time of exposure to darkness	Mg. eq. of malic acid formed	Mg. CO_2 evolved	Mg. CO_2 required by Thunberg reaction
Bryophyllum calycinum (Wolf(55))*.			
17.30–8	0·97	0·0	42·6
17.0–9	0·34	5·5	14·9
17.30–9	0·50	1·0	22·0
15.30–9	1·1	5·0	48·4
Sedum praealtum detached leaves (Bennet-Clark(7))†			
18–23	14·2	76·0	625·0
20–24	8·3	59·0	365·0

* Quantities expressed per 1 grm. dry weight.
† Quantities expressed per 100 grm. fresh weight.

lysis. Formic acid, however, does not accumulate in the tissues. Traces of it have been reported to be present in succulent plant tissues in some of the older work (Purjewitsch(39)), but Wolf has been unable to detect it. If it is formed it must, therefore, be converted into some other compound, and this other compound cannot be CO_2, as is shown by the results given in Table I. Oxalic acid is not present, so that if the formic acid is oxidised to this acid, it (the oxalic acid) must be caused to disappear by being reduced to an aldehyde which may undergo further change to other products.

It is, at all events, clear that if the Toeniessen-Brinkman reaction occurs, some acid (formic or oxalic) is reduced to an aldehyde and is then further converted into other products.

It has already been pointed out that the various 4-C acids are probably readily convertible into each other in living tissues and the nature of the changes has been discussed (Part I, p. 56). Direct evidence of the occurrence of these changes in succulent plants is not actually available, but there is no reason to suppose that such changes do not occur.

(b) *Altered carboxylase activity in succulent plants*

Ruhland and Wetzel(45) and Wolf(55) believe that the abnormal accumulation of 4-C acids is due to the diversion of pyruvic acid from the normal course of decarboxylation into a side reaction as indicated on p. 136. The cause of this is supposed to be the inhibition of carboxylase by acetaldehyde.

Wetzel[54] has shown that acetaldehyde does actually inhibit carboxylase activity. This is shown by the following results: a dried yeast preparation + pyruvic acid was mixed with acetaldehyde, and the CO_2 output during a given time was measured.

<div align="center">

TABLE II

The inhibition of carboxylase by aldehyde

</div>

Aldehyde added	Total aldehyde concentration $\%$	CO_2 output. Mg. per 1 grm. yeast per hour
0	0·018	638
0·01	0·027	583
0·05	0·06	330
0·10	0·109	298
1·0	1·004	105
2·0	2·001	11

Boklund has shown[12] that various amino compounds, such as aniline for example, catalyse the decarboxylation of pyruvic acid, and has suggested that the sequence of changes involved in this catalysis is

$$CH_3.CO.COOH + R.NH_2 \rightarrow CH_3.C\overset{\displaystyle OH}{\underset{\displaystyle NH.R}{—}}COOH \rightarrow CH_3.CH:O \quad \begin{array}{l} +CO_2 \\ \\ +RNH_2 \end{array}$$
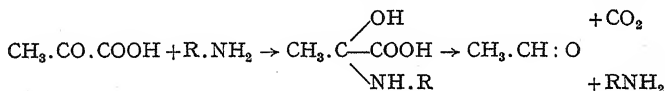
Since amines combine with aldehydes to form "Schiffs bases" thus:

$$CH_3.CH:O + H_2N.R = CH_3.CH:N.R + H_2O,$$
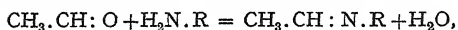
it is suggested that the inhibiting effect of aldehyde on carboxylase is due to the combination of aldehyde with the active amino groups which are supposed to be the cause of the catalytic activity of carboxylase.

It will be noted that a concentration of 0·05–0·1 per cent. roughly halves the activity of carboxylase.

Ruhland and Wetzel state that Kakesita[26] found concentrations of acetaldehyde of as much as 10·9 mg. per 10 c.c. of sap in the leaves of *Bryophyllum* (about 0·1 per cent.). The contention that the carboxylase activity in succulents may be reduced abnormally by these amounts of aldehyde seemed possible in view of this fact.

<div align="center">

(c) The aldehyde content of succulent leaves

</div>

Actually Ruhland and Wetzel have quoted the result of a single special experiment as though it were a typical result. In general Kakesita's results are not at all favourable to the Leipzig hypothesis. It is true that in one experiment he found an aldehyde content of

0·109 per cent. as stated by Ruhland and Wetzel, but the leaves had been kept under anaerobic conditions. Kakesita emphasises particularly that under normal conditions mature leaves have an aldehyde content of at most 0·017 per cent. On cutting the leaves the aldehyde content rises to the maximum value of about 0·06 per cent. attained at the 24th hour after cutting, after which a slow decrease in aldehyde content occurs.

This shows that under normal conditions the aldehyde content of *Bryophyllum* leaves is no greater than that of normal tissues, or of fermenting media (cf. Wetzel's figures in Table II).

It seems possible that Kakesita's method of estimating aldehyde might not be strictly accurate when applied to crude succulent plant saps. He added hydroxylamine sulphate to the sap and titrated the sulphuric acid which was set free as a result of the combination of the hydroxylamine base with aldehyde to give the oxime. Under these conditions many substances besides aldehyde might cause an acid reaction to develop in hydroxylamine sulphate solutions.

The present writer developed a nephelometric method for the estimation of such small concentrations of acetaldehyde as are present, based on the remarkably low solubility of the 2 : 4-dinitrophenyl-hydrazone of acetaldehyde. Concentrations of acetaldehyde between 0·05 and 0·005 per cent. can be readily estimated nephelometrically by simple addition of a solution of 2 : 4-dinitrophenylhydrazine in $2N$ hydrochloric acid to the solution to be tested, and comparing the turbidity with standard solutions of aldehyde.

When the concentration of aldehyde exceeds 0·05 per cent. the hydrazone forms too bulky and flocculent a precipitate to permit of nephelometric estimation. Artificial addition of acetaldehyde showed that the precipitation of the hydrazone is not hindered by colloidal or other substances present in the sap.

In none of the succulent plants examined were concentrations of aldehyde above 0·01 per cent. found. Sap samples artificially enriched with aldehyde so that the aldehyde content was 0·1 per cent. yielded abundant precipitates of the dinitrophenylhydrazone. Normal saps yielded a faint turbidity which was more orange than that obtained with pure aldehyde solutions. Typical results of the aldehyde contents obtained by the writer are given here: *Bryophyllum caly-cinum*, 0·01 per cent.; *Crassula arborescens*, 0·007 per cent.; *Stapelia variegata*, 0·007 per cent.; *Sedum praealtum* at 20° C., 0·006 per cent.; *Sedum praealtum* kept at 2–3° C., no turbidity, i.e. aldehyde content below 0·003 per cent.

It was repeatedly found that the aldehyde concentrations were too low to be detectable in the cooled tissues; it should be noted that acid accumulates much more vigorously in cooled tissues than in those kept at ordinary room temperatures. On the whole the results are not very divergent from Kakesita's. The aldehyde contents found are about one-third as great as those found by Kakesita, and there is some doubt if the aldehyde is acetaldehyde. Neither Kakesita's nor the writer's results afford support for the hypothesis that the carboxylatic activities of succulents are inhibited by an aldehyde.

(d) Relations between the quantity of sugar used and of malic acid formed

Great difficulty surrounds the problem of the mode of conversion of carbohydrate into malic acid. The first reaction given below does not occur: the second, the Toeniessen-Brinkman reaction, if it occurs, involves the further conversion of formic acid into unknown products by the process of reduction of the —COOH group to the aldehyde group —CH : O, since no acid other than malic is present: the third reaction merely records the necessity of considering the possibility that other compounds than formic acid might be formed; each molecule of malic acid is accompanied by two atoms of "other carbon": finally the fourth reaction should be considered.

$$\text{I.} \quad C_6H_{12}O_6 \rightarrow C_4H_6O_5 + 2CO_2$$
$$\text{II.} \quad C_6H_{12}O_6 \rightarrow C_4H_6O_5 + 2H.COOH$$
$$\text{III.} \quad C_6H_{12}O_6 \rightarrow C_4H_6O_5 + C_2X$$
$$\text{IV.} \quad 2C_6H_{12}O_6 \rightarrow 3C_4H_6O_5$$
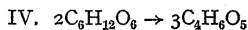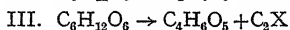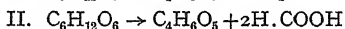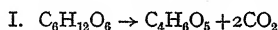
Obviously it is important to find out how many molecules of acid are formed for each molecule of sugar which disappears. The writer started to investigate this question some years ago, but the results only serve to increase the complexity of the problem.

It was noted that the reducing sugar content of the sap of *Sedum praealtum* decreased about 50 per cent. when the sap was warmed with 1 per cent. HCl for a short time, instead of increasing owing to hydrolysis of di- or polysaccharides. This was found to be due to the presence of large quantities of a heptose sugar. This sugar, sedoheptose, had been discovered by La Forge and Hudson [30] in the leaves of *Sedum spectabile*. They showed that it was a 2-ketoheptose, which had the peculiarity, that warming with dilute mineral acids converts it into an anhydro-compound which does not reduce alkaline copper solutions.

La Forge later showed[31] that its configuration differs from that of the common naturally occurring sugars in that all the hydroxyl groups are probably on the same side of the molecule. The configuration has since been definitely established, and the positions of the oxide rings determined by Hibbert and Anderson[24]. The formulae both of sedoheptose and of the anhydro-derivative sedosan are given below, written in the conventional straight chain manner, and also so as to show the spatial arrangement of the atoms.

Sedoheptose   Sedosan

It will be noted that sedosan possesses no ketonic hydroxyl group and therefore does not reduce alkaline copper or ferricyanide solutions. Warming with weak acids causes the equilibrium noted above to be established in which about 80 per cent. of the sugar is present as sedosan and 20 per cent. as the free sugar. The cause of this remarkable reaction is the close proximity of the hydroxyl groups marked with asterisks. This reaction has been used by the writer for the purpose of estimating sedoheptose in succulent plant saps.

Warming in a boiling water-bath with 1 per cent. HCl for 15 min. causes the copper reduction of a sedoheptose solution to be decreased 75 per cent., so that equilibrium is almost attained. A slight decrease occurs in the reducing power of fructose solutions (about 5 per cent.) with such a short period of heating. The change in copper reduction brought about by this acid treatment, after removal of disaccharides by enzymes, is therefore a measure of the sedoheptose content. It is, however, made somewhat inaccurate when other ketoses are present.

The curious result was obtained that the aldose content (measured by oxidation with alkaline iodine) + sedoheptose content (estimated

as above) was equal to the total monosaccharide reducing sugar. Fructose and other ketoses are therefore absent, or only present in traces. Sucrose was not detected with certainty; the mode of estimation used was inversion with invertase. The pentose content was about 10 per cent. of the total aldose content.

Remarkable fluctuations in the sedoheptose content occur during the course of the day and night. The results of a typical experiment in which leaves of *Sedum praealtum* were used are given graphically in Fig. 3. The times of day are shown as abscissae, and the ordinates
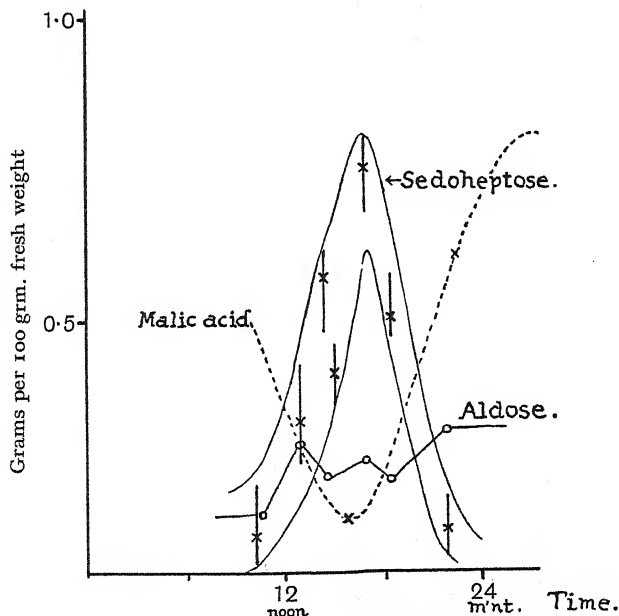
Fig. 3. The diurnal fluctuation in acid and sugar content of detached leaves of *Sedum praealtum* on June 20th.

give the percentages of sedoheptose and of aldose at various times. It will be noted that little change in aldose content occurs, the concentration is about 0·2 per cent. of the fresh weight. The sedoheptose content rises from less than 0·1 per cent. at 10 a.m. to 0·8 per cent. at 6 p.m. and falls off rapidly to a value of about 0·1 per cent. at midnight. The sedoheptose content falls whilst the malic acid content of the leaves rises. The maximum sedoheptose and minimum malic acid content occur at about 6 p.m.

In this particular experiment, between 5 and 10.30 p.m., 0·7 grm. of sedoheptose disappeared, and 8·0 mg. eq. (0·536 grm.) of malic

acid accumulated per 100 grm. fresh weight. The CO_2 output of another sample of similar leaves kept under identical conditions was 39 mg. per 100 grm. fresh weight. It is convenient to express these quantities in terms of the carbon content of the substances:

Quantity of sedoheptose lost contains 280 mg. of carbon.
     ,,   malic acid formed contains 190 mg. of carbon.
     ,, CO_2 evolved contains 10·6 mg. of carbon.
     ,,   aldose, no change.

It will be noted that for 280 mg. of sedoheptose carbon lost, 200 mg. of malic acid and CO_2 carbon are recovered, leaving 80 mg. or two-sevenths of the total carbon loss unaccounted for. The temptation, not perhaps justified by the present evidence, is to suppose that four-sevenths of the sedoheptose carbon (and not five-sevenths as this experiment indicates) is converted into malic acid, and that three-sevenths is converted into other products, possibly starch, since the starch content of the leaves rises. This would involve the occurrence of the reactions:

$$C_7H_{14}O_7 \rightarrow C_4H_6O_5 + C_3X; \quad C_3X \rightarrow \text{polysaccharide.}$$
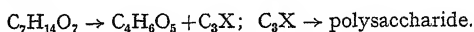
Wolf's results are not in close agreement with those described above, but direct comparison cannot be made as Wolf only records a single analysis at 5 p.m. and another at 9 a.m., and not a series of analyses. Furthermore, the complicating effects of the presence of sedoheptose were not realised.

TABLE III

Changes in sugar and acid content of Bryophyllum *leaves during the night (Wolf)*

Milligram-mols per 1 grm. dry weight

Decrease in fermentable sugar	Decrease in non-fermentable sugar (sedoheptose?)	Increase in malic acid	Temperature °C.
0·065	0·253	0·210	8
0·275	0·225	0·50	20
0·195	0·233	0·22	35

Sedoheptose is not fermentable by common yeast; Wolf's results seem to indicate that both sedoheptose and the other sugars contribute to the malic acid formation: the former is the chief contributor in the first experiment (8° C.), and in the others the fermentable sugar contributes also to acid formation apparently. The results agree with the writer's in showing that one, or less than one molecule, of acid is formed for each molecule of sugar used.

(e) Malic acid disappearance in darkness. The Leipzig hypothesis

Ruhland, Wetzel, and Wolf assume that malic acid is oxidised in the tissues to oxalacetic acid in the manner indicated in Part I, p. 56. The oxalacetic acid is supposed to be converted into pyruvic acid in the ordinary way. Hahn and Haarmann (21, 22) have shown that muscle tissue can bring about the oxidation of malic acid to pyruvic acid (probably through the stage of oxalacetic acid) if semicarbazide is present; the latter fixes the pyruvic acid formed as its semicarbazone.

The complete series of transformations which are involved in the formation and disappearance of malic acid are, therefore, supposed to be

Sugar
↓
Methyl-glyoxal   Formic acid
$(CH_3.CO.CH:O)$ $(H.COOH)$
↓   Diketoadipic acid   →   +
Pyruvic acid ↗ $(COOH.CO.CH_2.CH_2.CO.COOH)$ Succinic acid
$(CH_3.CO.COOH)$ $(COOH.CH_2.CH_2:$
↓   ↖   ↓ $COOH)$
Aldehyde   Oxalacetic acid   ←   Malic acid
$(CH_3.CH:O)$ $(COOH.CO.CH_2.COOH)$ $(COOH.CHOH.CH_2.$
  $+CO_2$ $COOH)$
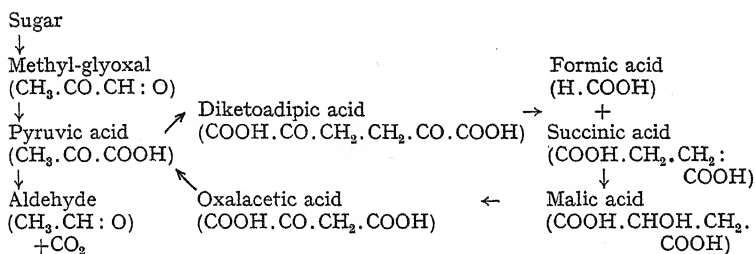
Accumulation of malic acid is supposed to be due to inhibition of the activity of carboxylase, and its final disappearance to be due to recovery of the activity of carboxylase. The changes in activity of carboxylase are supposed to be brought about by changes in the aldehyde content of the leaves (Ruhland and Wetzel (45)).

The aldehyde concentrations are, however, almost certainly too low to produce marked inhibition of carboxylase. Kakesita's results also show that the aldehyde concentration rises, when leaves are cut from the plant, to a maximum value attained about the 24th hour after cutting. At first, when the aldehyde content is low malic acid accumulates; 24 hours later the accumulated acid has commenced to disappear yet the aldehyde concentration has now attained its greatest value. These relationships between aldehyde content and the acidity changes are the exact opposite of the requirements of the Leipzig theory.

(f) Malic acid disappearance in light

Mayer (34, 35) first showed that lighted leaves of members of the Crassulaceae evolve oxygen when enclosed in atmospheres totally devoid of carbon dioxide, and malic acid disappeared concurrently. The change $3C_4H_6O_5 + 3H_2O = 2C_6H_{12}O_6 + 3O_2$ is supposed to occur.

It was not possible to decide whether malic acid was first converted into CO_2 which was photosynthesised, or whether some direct photo-synthesis of malic acid occurred. Mayer suggested that oxygen could be photochemically split off from a carboxyl group, and the active hydroxy-aldehydes formed might condense to give sugar.

Spoehr[48] showed that complete photochemical oxidation of malic acid could occur *in vitro*, and he believed that this process occurred in succulent plants and that the CO_2 so formed in the tissues was photosynthesised. Spoehr's view that the process is a purely photochemical breakdown unconnected with the enzymes or organisation of the cell has been mentioned; the fact that narcotics prevent photo-deacidification, however, shows that the Spoehr reaction cannot be the mode of acid breakdown occurring in the normal living plant. Wolf brought forward additional evidence against the view that the Spoehr reaction occurred *in vivo*. He showed that the laevo acid disappeared more rapidly than the dextro acid, which makes it seem likely that an optically active enzyme is involved in the disappearance of the acid: he showed, moreover, that the temperature coefficient of the photo-deacidification was of the order of magnitude of a chemical rather than of a photochemical change (the observed Q_{10} was between 2 and 3).

Wolf supposes that the same chemical changes take place, when the acid disappears in lighted leaves, as occur when it disappears from darkened leaves. Malic acid is supposed to be converted into, first oxalacetic, and then pyruvic acid, which gives rise to CO_2 and aldehyde, both of which are supposed by him to be converted eventually into carbohydrate.

Warburg[53] and Astruč[3] both showed that carbon dioxide inhibited the process of deacidification in light in a manner similar to the inhibition caused by anaesthetics. Wolf has confirmed and extended these results. As a result he considers that the accelerated disappearance of malic acid from lighted leaves is due to the removal of CO_2 by photosynthesis. The inhibition caused by carbon dioxide is thought by Wolf to be due to the fact that it induces a condition of narcosis in which the respiratory phenomena to some extent resemble those occurring under anaerobic conditions. Thomas showed[51] that under such conditions some alcohol and considerable quantities of acetaldehyde accumulate in the tissues of apples. Wolf considers that similar effects will occur in Crassulacean leaves, and that the accumulation of aldehyde will cause inhibition of carboxylase as indicated in section (*b*), and that the converse is also true.

(g) *Summary of the Leipzig hypothesis*

The main features of the hypothesis of Ruhland, Wetzel, and Wolf are as follows. Pyruvic acid is formed from sugars by the zymase complex of enzymes: its normal conversion into aldehyde and carbon dioxide is inhibited by the abnormally high aldehyde content of the tissue, and it is consequently converted into malic acid. Such a conversion of a 3-C into a 4-C compound necessarily involves the accumulation of 1-C or 2-C compounds or their conversion into complex compounds. Actually 1-C and 2-C compounds do not accumulate, nor are they excreted; so conversion into complex compounds must occur. Such anabolic syntheses are frequently inhibited or stopped by anaesthetics and by anaerobic conditions; these hypothetical compounds might consequently accumulate under such conditions. This possibility is at present being investigated.

The abnormal aldehyde concentrations of succulent plant tissues which are postulated, are thought to be caused in part by the CO_2 exercising a narcotising effect on the respiratory system. When the plants are illuminated, photosynthesis reduces the CO_2 concentration; in consequence of this the aldehyde content is supposed to be reduced and the increased carboxylatic activity of the tissue causes the acid to disappear.

(h) *The acid reduction hypothesis*

The present writer (7, 8) put forward a different view of the processes occurring in Crassulacean metabolism. It was supposed that a process of glycolysis occurred essentially comparable to that occurring in non-succulent tissues, and that part of the carbon of these products of glycolysis was evolved as CO_2, but that the greater part underwent a process of oxidative anabolism comparable to the processes which have been shown to occur in apple fruits (Blackman (10)), the oxidative rebuilding of alcohol to glycogen in yeast (Lundin (32), Meyerhof (36)), and the lactic acid-glycogen conversion in muscle. Malic acid was assumed to be an intermediate product in the conversion of the products of glycolysis into carbohydrate. Its accumulation was supposed to be due to the velocity constant of its formation exceeding that of its further conversion, as suggested on p. 134.

Precise details of the chemical changes involved were not suggested, as the evidence then available did not justify such speculation. The view was, however, put forward that disappearance of the acid from the tissue is brought about by reduction of the acid, and not by

its oxidation. Three main lines of evidence are held by the writer to support these claims, and these will be considered in the subsequent sections.

(i) *The effects of continued starvation on Crassulacean leaves*

Continuous loss of carbon (as CO_2) takes place from detached leaves placed in darkness. Characteristic changes occur in the rate of carbon dioxide emission, and in the acid content of the tissues as this starvation progresses. The results of a typical experiment are indicated graphically in Fig. 4.
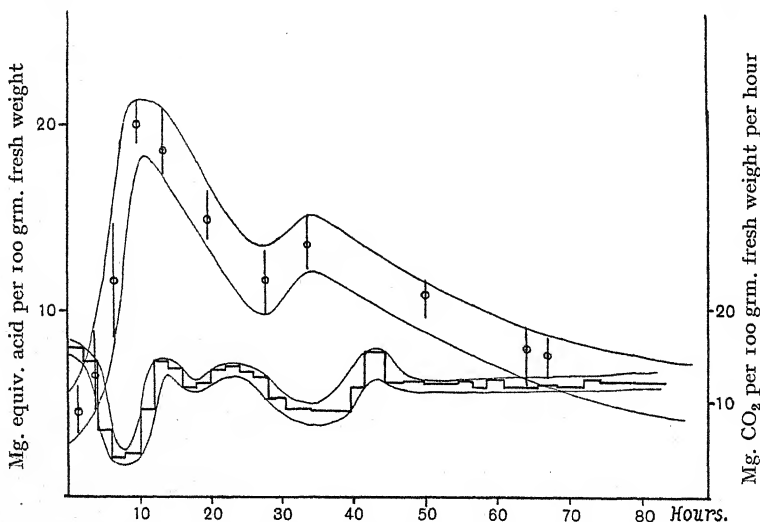
Fig. 4. Rate of respiration (lower curve) and acid content (upper curve) of detached leaves of *Sedum praealtum* placed in darkness at 8 p.m. on June 17th, and kept in continued darkness at 21° C. (Bennet-Clark (8)).

Records of the rate of CO_2 output and of the acid content of the leaves of *Sedum praealtum* are shown as ordinates in Fig. 4[1]. The leaves of which the acid contents were estimated were exactly similar to those of which the CO_2 output was recorded, and were collected from the same plant and kept under identical conditions during the starvation period.

[1] Each record of the titratable acidity is the average of four separate estimations. The maximum and minimum values are shown as the tops and bottoms of vertical lines drawn through the points which show the average value. CO_2 estimations were carried out by the Pettenkofer method.

The rate of CO_2 output is high immediately after the leaves are placed in darkness (at 8 p.m. on June 16th), but very rapidly the rate falls to about a quarter of the initial value. Thus the rate of CO_2 output falls off rapidly concurrently with a rapid increase in malic acid content. After some fluctuation the rate eventually attains a relatively constant value. It is convenient to refer to this final phase of the starvation as the equilibrium phase. The leaves eventually become yellow and senescent, but this senescence is not associated with marked change in rate of CO_2 output.

This experiment does not show as clearly as some others that the malic acid content rises to two or more peak values. The decrease in acidity is followed by a secondary rise in acidity, which is fairly clearly indicated. The second maximum in the curve is followed by a steady decrease in acidity of roughly exponential form. It is important to note that the maxima on the acidity-time curve do not coincide with maxima on the CO_2 output-time curve. They nearly, but not exactly, coincide with the minima on the CO_2 output-time curve. Similar results have been obtained in all experiments with both *Sedum praealtum* and *Crassula lactea*, the two species examined.

These results indicate that malic acid is not the substrate from which carbon dioxide is formed. If it were, the rate of CO_2 output should be proportional to the concentration of malic acid. The reactions postulated by some of the older investigators (de Vries, Nathansohn, Purjewitsch, and others), shown as I below, and the reactions postulated by the Leipzig school, shown as II, therefore appear improbable unless they account for only a very small fraction of the total carbon dioxide output.

I. $C_4H_6O_5 + 3O_2 = 4CO_2 + 3H_2O$.
II. $C_4H_6O_5 \rightarrow COOH.CH_2.CO.COOH \rightarrow CH_3.CO.COOH + CO_2$
$CH_3.CO.COOH \rightarrow CH_3.CH:O + CO_2$.
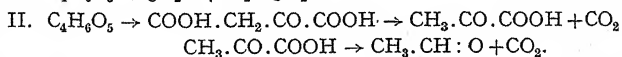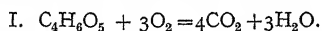
Since pyruvic and oxalacetic acids do not accumulate, the rate of CO_2 output in the second case must also be proportional to the malic acid concentration unless drastic fluctuations occur in the velocity constants of the reactions.

In order to give a clear picture of the quantity of CO_2 which might be expected to be formed from malic acid, the results of the same experiment are graphically illustrated in another manner. In Fig. 5 instead of giving the acid contents of the tissue, the rate of change of acidity is given and is expressed in terms of the quantity of carbon present in the acid. Gain in acidity is shown by ordinates below the origin and losses of acid and carbon dioxide are shown by ordinates

above the origin. These gains and losses are expressed as mg. carbon per hour per 100 grm. fresh weight (1 mg. eq. of malic acid per hour is of course 24 mg. carbon per hour). Note that the acid loss-time curve crosses the zero line at the times corresponding with the maxima and minima of the acidity-time curve.
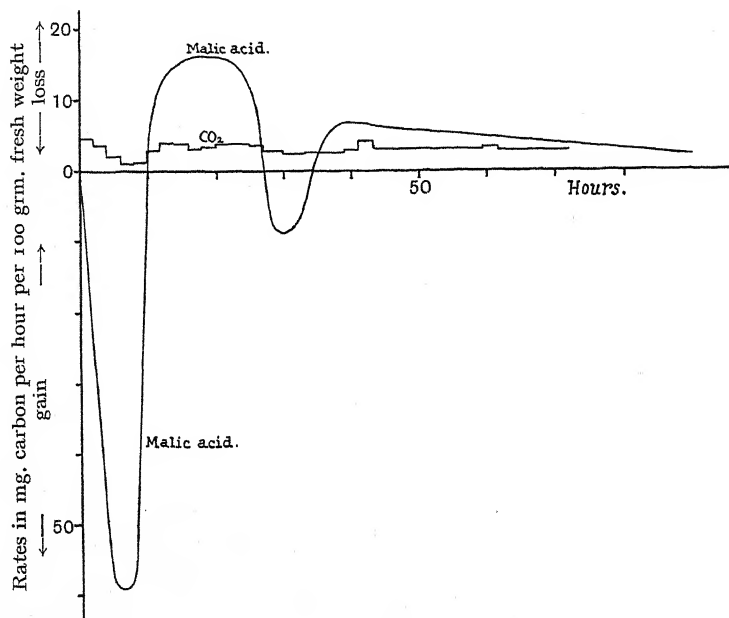
Fig. 5. Net rates of malic acid accumulation and disappearance, and of CO_2 output, expressed in terms of the carbon contents. *Sedum praealtum* leaves, same experiment as in Fig. 3.

The most important feature of the whole experiment is that for a considerable period of the starvation time, but most noticeably between the 11th and 26th hours, the rate at which carbon disappears from the form of malic acid exceeds the rate at which carbon is evolved as carbon dioxide. It therefore follows as an inevitable consequence that the malic acid or part of the malic acid is stored up in the tissue in some neutral form. Between the 15th and 21st hours the rate of malic acid carbon loss is 16 mg. per hour, and the average rate of CO_2 carbon output is 3·3 mg. per hour. If the Leipzig reactions (II, on p. 49) occurred at least 8 mg. of CO_2 carbon should be evolved per hour. If the reaction given below occurred:

$$COOH.CH_2.CHOH.COOH \rightarrow CO_2 + C_3X,$$
$$C_3X \rightarrow \text{carbohydrate},$$
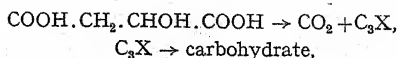
at least 4 mg. of CO_2 carbon per hour should be evolved. It is important to note that these figures (8 and 4 mg. per hour) are *minimum* quantities which could be evolved on the basis of the Leipzig reactions and the imaginary reactions just given. Actually larger quantities would be expected, since the malic acid-carbon loss curve given in Fig. 5 represents the *net* rate of disappearance of acid and not the *gross* rate. It is almost certain that formation and disappearance of acid both proceed continuously, and therefore the gross rates of acid formation or disappearance must exceed the net rates. Since the net rate of acid carbon disappearance is more than four times the CO_2 carbon output, it follows that the loss of acidity is not due to carbon dioxide splitting from the carboxyl groups.

Other processes by which the acid properties of the carboxyl group might be removed are: (1) conversion into an ester or anhydride, (2) conversion into amide, or ammonium salt, (3) reduction to an aldehyde group. The first two probably do not occur to any considerable extent, since amides and ammonium salts are only found in small quantity, and anhydrides and esters are also only present in traces. One is therefore led, by exclusion of these other possibilities, to the view that the carboxyl groups are reduced to aldehyde groups. The evidence does not permit one to assume that malic acid is reduced to malic aldehyde. Another acid or other acids (glycollic acid, for example) might be formed from malic acid, and be converted into an aldehyde or aldehydes, and the possibility of conversion of such hydroxy-aldehydes to carbohydrates is not very remote.

The evidence given in the next section indicates further that the loss of acid, i.e. the loss of carboxyl groups, is due to reduction rather than to interaction with ammonia or hydroxyl groups with ester or anhydride formation.

(j) The significance of the respiratory quotients

Since the time of de Saussure[15], it has been known that the values of the ratio CO_2/O_2 differ strikingly from unity in succulent plants. Whilst malic acid is accumulating, the R.Q. has a very low value (often as low as 0·1), since oxygen is consumed in the conversion of sugars to malic acid without any corresponding output of CO_2. During the phase of malic acid disappearance the value of the R.Q. is, conversely, higher than unity. Its exact values are of great interest.

Astruč[3] gives many records of the value of the R.Q. during the phase of acid disappearance; in many cases the values recorded by him are between 1·00 and 1·33, but occasionally higher values were

recorded. The following values are taken from his paper: 1·50, 1·48, 1·53, 1·75, 1·78, 2·41, 1·42. Astruč makes no comment on their significance.

When malic acid is completely oxidised to CO_2 and water, the ratio CO_2/O_2 is 1·33. If both malic acid and some other substrate (e.g. sugar) were oxidised the R.Q. would be less than 1·33. The high values found by Astruč could be explained either by the partial oxidation of the malic acid, or by the conversion of part or all of the malic acid carbon into carbohydrate and the storage of this carbohydrate in the tissues.

On the basis of Ruhland and Wetzel's views one might expect the following reactions to occur:

$$C_4H_6O_5 \quad + \tfrac{1}{2}O_2 = 2CO_2 + H_2O + CH_3 CH : O,$$
$$CH_3 . CH : O + \tfrac{1}{2}O_2 = \tfrac{1}{3}(C_6H_{12}O_6).$$
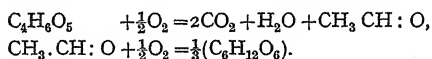
If those reactions occurred and none of the sugar formed was oxidised the R.Q. would be 2·0, but if some of the sugar was oxidised it would be less than 2·0. In no circumstances would it be more than 2·0. Astruč records two cases in which the values of the R.Q. were 2·39 and 2·41, and the present writer has also obtained values higher than 2·0. Such high values might be explained in three ways.

(1) The aldehyde assumed to be formed by the Leipzig school might escape as vapour from the respiring tissues, and either entirely or in part escape oxidation into carbohydrate in consequence. The writer has shown that this does not occur by bubbling the gas stream which left the respiring leaves through a saturated solution of 2 : 4-dinitrophenylhydrazine in $2N$ HCl; no precipitate was formed, whereas an abundant precipitate of the aldehyde hydrazone formed in a control where an air stream was led over a 0·1 per cent. solution of aldehyde and then into the dinitrophenylhydrazine reagent.

(2) On the Leipzig view, half or less than half of the acid might be converted into sugar. If, however, more than half were converted into carbohydrate the R.Q. would exceed 2·0. This is the view which was introduced in the last section (section (*i*)), and as pointed out there, if this happens, it follows that carboxyl groups are reduced to aldehyde groups.

(3) The results might be due to experimental errors, or erroneous interpretations of the analyses. Maquenne and Demoussy explained high values of the R.Q. by supposing that carbon dioxide came out of solution in the tissue fluids, and that the CO_2 output observed consisted of CO_2 formed in respiration and also of CO_2 which came out of

solution. The latter would only occur if the temperature or the partial pressure of CO_2 in the surrounding atmosphere changed. It is not clear that a rise in temperature would cause the CO_2 to come out of solution in the tissues. It has been shown that the partial pressure of CO_2 in the internal atmosphere of apples is raised by a rise in temperature (Ekambaram; Thesis, Cambridge University, quoted by Blackman and Parija (11)). The rise in CO_2 tension tends to force more of the gas into solution, and this tendency is balanced by the lower solubility of the gas at the higher temperature. In such a case as the Crassulacean leaf where the compositions of the internal atmospheres are quite unknown, it is not possible to predict whether rise in temperature will cause CO_2 to pass into solution or come out of solution. Indirect evidence suggests that the former is the case. If this be accepted Maquenne and Demoussy's explanation of the high initial value of the R.Q.'s of succulents is not valid.

In the present writer's work (9) continuous records were obtained of the R.Q. by a method in which the leaves are kept under constant conditions of temperature and external CO_2 tension. High initial values of the R.Q. were found; during the phase of acid accumulation values much lower than unity were obtained, and these were succeeded by values higher than 2·0 during the phase of acid disappearance. Since the plants had been in equilibrium with their environment from the start of the experiment, the high values of the R.Q. obtained at the end are clearly not due to CO_2 coming out of solution. In this case therefore the only explanation of the result is that more than half of the acid is reduced and possibly converted into carbohydrate.

(k) *The effects of change of temperature*

The evidence discussed in the last two sections suggests that the scheme of reactions given below represents the position of malic acid in Crassulacean metabolism:

$$\text{Active sugar} \quad \leftarrow \quad \text{carbohydrate}$$
$$\downarrow \qquad\qquad\qquad \uparrow$$
$$\text{Products of glycolysis} \rightarrow \text{malic acid}$$
$$\downarrow$$
$$CO_2$$
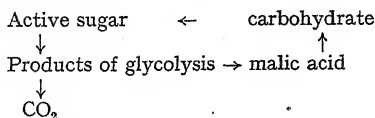
This agrees with the view that the malic acid is reduced, and is in agreement with the fact that the rate of CO_2 output is not proportional to the malic acid concentration. The initial rate of CO_2 production is found to be high, which doubtless corresponds with the initial high sugar concentration at the beginning of the starvation period.

This cycle of changes may be written in the simplified form

$$\text{Carbohydrate} \rightleftharpoons \text{malic acid} + \text{energy}$$
$$\downarrow$$
$$CO_2$$
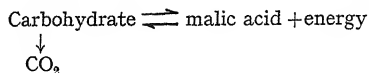
Such an equilibrium should conform to the Le Chatelier principle, and be displaced in the direction in which energy is absorbed by rise in temperature. The evidence suggests that this is the case[1].

Detached leaves of *Sedum praealtum* kept at a high temperature in darkness soon lose the acid which they first accumulate, and the acidity is maintained at a low value as shown by the curve *Oc* 15*a* in Fig. 6. Exactly similar leaves kept at 3° C. accumulate acid and
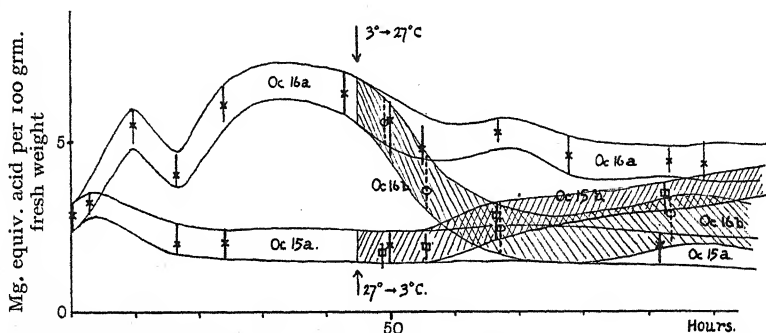
Fig. 6. Acidities of *Sedum praealtum* placed in darkness on October 15th. *Oc* 15*a* at 27° C.; *Oc* 16*a* at 3° C.; *Oc* 15*b* changed from 27° to 3° C.; *Oc* 16*b* changed from 3° to 27° C. at 45th hour after placing in darkness.

eventually maintain a roughly constant high equilibrium acidity as shown by the curve *Oc* 16*a*. Changing the leaves from the low to the high temperature causes the acid to disappear (curve *Oc* 16*b*), and conversely changing the leaves from the high to the low temperature (curve *Oc* 15*b*) causes the acid concentration to rise.

Indirect evidence indicates that the malic acid which disappears when the cool leaves are transferred to the high temperature is converted into carbohydrate. The CO_2 outputs of leaves exactly similar to those referred to in records *Oc* 15*a*, 15*b*, 16*a*, and 16*b* are given in Fig. 7. The record *Oc* 15 gives the rates of CO_2 output of leaves kept at 27° C. and then transferred to another thermostat kept at 3° C.

[1] Wolf's statement(55) that higher acidities are attained at 20° C. than at 10° C. and lower temperatures is based on a measurement during the early stage of the starvation. The acidity rises later to the equilibrium value which is higher the lower the temperature.

Record *Oc* 16 similarly shows the respiration rates of leaves kept at first at 3° C., and then transferred to the 27° C. thermostat.

The equilibrium rate of respiration at 27° C. (shown by records *Oc* 15 and 16, and by the dotted line) is maintained without the leaves losing malic acid (see *Oc* 15*a*), so that evidently this CO_2 is supplied by the carbohydrate reserves of the leaves. The excess CO_2 output formed when the cooled leaves are warmed (the part of record *Oc* 16 above the dotted line) is evidently produced directly or indirectly from the malic acid which disappears at the same time.
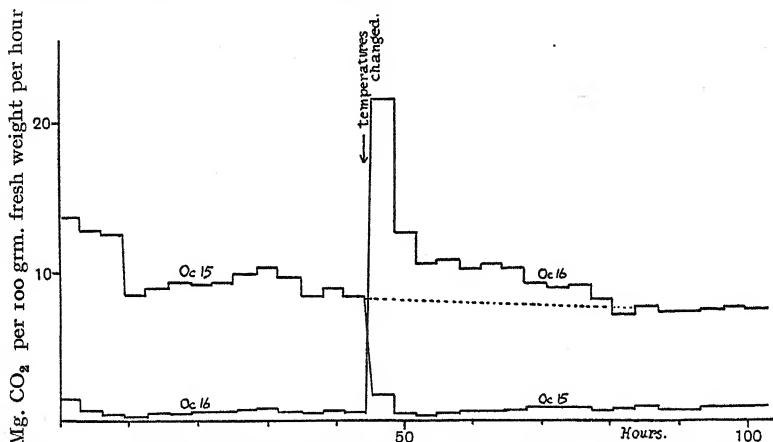
Fig. 7. The CO_2 outputs of similar leaves to those of Fig. 6. *Oc* 15 in darkness first at 27° C. but changed to 3° C. at the 45th hour. *Oc* 16 at 3° C. changed to 27° C. at 45th hour (Bennet-Clark (7, 8)).

The amount of malic acid carbon which is lost from the leaves on warming is 90 mg. per 100 grm. fresh weight (3·75 mg. eq.), and the quantity of excess carbon dioxide which is evolved is 118 mg., which contains only 32 mg. of carbon. So the loss of malic acid is clearly not due to its conversion into carbon dioxide, or at most one-third of it is converted into carbon dioxide.

The simplest explanation of the phenomenon is that the malic acid which disappears is converted into sugar, and the resultant rise in sugar concentration causes the rate of carbon dioxide output to be greater than the expected equilibrium rate. Thus one does not expect a definite stoichiometric ratio between the amount of CO_2 evolved and the amount of acid which disappears: the relative quantities of the two which are found experimentally are very variable, but in all cases the amount of CO_2 carbon evolved is less than the amount of malic acid carbon which disappears.

The expectations based on our hypothesis and the Le Chatelier principle are consequently realised.

(l) *The acid reduction hypothesis summarised*

The expectations based on this hypothesis are briefly as follows: Immediately after darkening cut leaves of a member of the Crassulaceae, the sugar content is high, and since the carbon dioxide is derived from products of glycolysis (see p. 153) the rate at which it is produced is high. The sugar concentration, and also this initial rate of respiration, decrease rapidly owing to conversion of sugar into malic acid. This latter accumulates, since the velocity of its formation exceeds that of its further conversion. This further conversion is
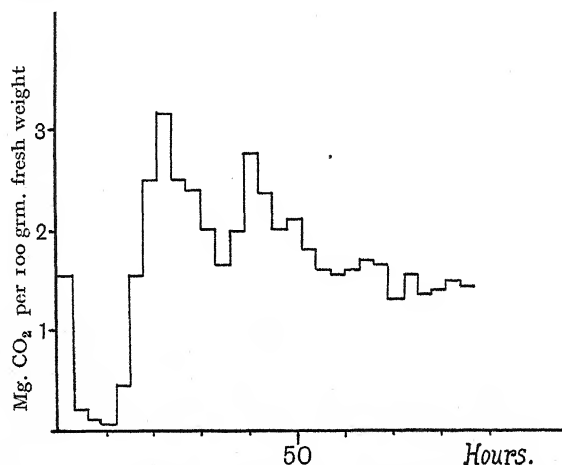
Fig. 8. CO_2 output of detached *Crassula lactea* leaves in continuous darkness at $21°$ C. Leaves placed in darkness at 5 p.m., May 28th.

brought about by the reduction of the acid, and polymerisation of the hydroxy-aldehydes so formed. This process is supposed to result in the formation of carbohydrates. The resultant rise in sugar concentration causes a secondary rise in CO_2 output. This occurs at the time that the malic acid disappears. This rise in sugar concentration causes a secondary rise in acid content, followed by a tertiary rise in sugar content and corresponding tertiary rise in rate of CO_2 output.

Thus rhythmic fluctuation in acidity and rate of CO_2 output should occur, and the two curves should be out of step. This is experimentally observed. Compare the results given in Fig. 4, and also the very striking periodicity in respiration rate of the *Crassula lactea* leaves kept in darkness at a constant temperature (Fig. 8). Note that rise in acidity is associated with decrease in respiration rate.

Deacidification of the leaves in light is due primarily in the writer's opinion to the high temperature of the illuminated leaves, and the operation of the Le Chatelier principle as discussed in the last section. It may be pointed out that it has been known for many years (Askenasy(2)) that the leaves of succulent plants when illuminated become much hotter than do ordinary thin leaves. At midday in March in Germany the temperatures of *Sempervivum* leaves, for example, were as high as 50–60° C. This may be due to lesser cooling of succulent leaves by transpiration; cf. also Huber(25).

The distribution of the typical Crassulacean metabolism throughout the plant kingdom is at present a matter of speculation rather than exact knowledge. One may provisionally guess that plants which show diurnal periodicity (see Part I, p. 66) possess the Crassulacean type of metabolism. In the next part evidence will be produced which indicates that the fungi also possess the power of reducing the carboxyl groups of organic acids.

(m) *The Liebig-Baur hypothesis of photosynthesis*

The remarkable feature of the reaction, the reduction of carboxyl to the aldehyde group, is that it requires an energy supply to bring it about. One must assume that this energy is supplied by some catabolic reaction which is coupled with the carboxyl-reducing reaction.

If one can accept the conclusions which have been drawn in the preceding sections, a new complexion is put on the old Liebig theory of photosynthesis. The view that CO_2 was first converted into oxalic acid, and thence into other acids and finally into sugar, was discarded by plant physiologists largely on account of the work of Mayer and de Vries on the acid metabolism of the Crassulaceae. It was argued that, since the organic acids disappeared in sunlight and reappeared when the leaves were darkened, they could not possibly be intermediate products in the building up of sugars in photosynthesis. The preceding discussion has shown that that argument is erroneous.

If the cell possesses a mechanism of coupled reactions by which carboxyl groups can be reduced, oxalic acid might be reduced and converted eventually to carbohydrate. It may be significant that Spoehr and McGee(50) claim to have shown that a close connection exists between respiration and the photosynthetic activities of tissues. Moreover Baur(5) has shown that under certain conditions the reaction

$$2CO_2 \rightleftharpoons (COO')_2$$
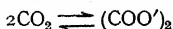
is reversible, and has suggested an imaginary model for the first stage

of photosynthesis based on this. Whilst these matters come within the realm of pure speculation at present, it should be borne in mind that the original objections to the Liebig theory have largely vanished.

C. General summary and conclusion

It may be well to attempt to summarise our present knowledge of Crassulacean metabolism. Evidence brought forward by Wolf and by the present writer clearly shows that no more than one molecule of malic acid is formed from each molecule of sugar. Therefore, for each molecule of sugar converted to acid two atoms of carbon (or three, if the sugar is sedoheptose) must be converted into some other substance or substances.

This carbon does not reappear as carbon dioxide. Accumulations of other acids are not found in the tissues of the Crassulaceae, so this missing carbon is not stored in the tissues as formic, oxalic, glycollic, glyoxylic or lactic acids. Only traces of volatile carbon compounds are present, and it therefore follows that no 1-C or 2-C compounds are present in the tissues in sufficient quantity to account for the missing carbon. Aldehydes and ketones containing three carbon atoms are present only in traces, since dinitrophenylhydrazine yields only a very slight precipitate with the sap. It seems likely in consequence that 1-, 2-, or 3-C compounds, formed simultaneously with malic acid from sugar, are built back again into polysaccharide.

Direct evidence also shows that considerable quantities of malic acid are converted (the writer considers that all the acid is so converted) into compounds other than carbon dioxide. The exclusion of the substances mentioned in the last paragraph again suggests that this acid is converted into polysaccharide.

There is little doubt therefore that the reactions involving sugars and malic acid are of the nature indicated below:

$$\text{Polysaccharide} \rightarrow C_6H_{12}O_6 \leftarrow \text{polysaccharide}$$

$$\overline{} \underset{\text{or } C_2X}{2CX} + C_4H_6O_5 \overline{}$$

or

$$\text{Polysaccharide} \rightarrow C_7H_{14}O_7 \leftarrow \text{polysaccharide}$$

$$\overline{} C_3X + C_4H_6O_5 \overline{}$$
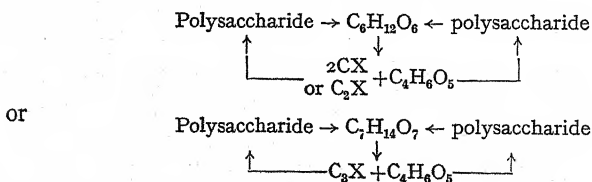
Carbon dioxide has been purposely omitted from these schemes of reactions, since different views as to its origin are held by the Leipzig school and the present writer. The former hold that it originates from oxalacetic acid and pyruvic acid formed by partial oxidation of the

malic acid. For reasons which have been detailed the writer thinks that it must originate from some intermediate product in the conversion of sugar into malic acid, and that loss of acidity is due to reaction I (below) rather than reaction II.

$$\text{I. } R.COOH - O \rightarrow R.CH:O,$$
$$\text{II. } R.COOH \rightarrow R.H + CO_2.$$
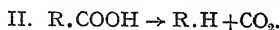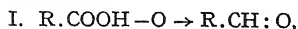
It is desirable to defer comparison of Crassulacean metabolism with the acid metabolism of other plants till the end of the third part of this review.

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REVIEWS

Thomas Johnson, Botanist and Royalist. By H. Wallis Kew and
    H. E. Powell. Longmans, Green and Co., Ltd., London, 1932.
    Pp. xi + 151, 10 text-figs. and 13 plates. Price 8s. 6d.

This attractively produced book is the first attempt to give a full account
of the life and work of one who may well be called "The Father of British
Field Botany." This neglect is no doubt largely due to the very scanty records
of his life which have survived. Such as there are, moreover, in many cases
confuse rather than help the biographer, owing to the popularity of the name
Thomas Johnson, and the consequent ambiguity of many entries under it in
Parish Registers and the Court Book of the Society of Apothecaries.

The authors are to be congratulated on producing a well-documented and
readable account of this "learned, amiable, brave man."

The first chapter is devoted to a list of Johnson's publications and of other
works to which the authors referred during their investigations. In this con-
nection it may be worth pointing out that, in addition to the *Opuscula* edited
by T. S. Ralph in 1847, there appeared, two years later, a separate reprint
of the *Descriptio Itineris...* of 1632, also edited by Ralph and published by
Pamplin. This is not mentioned in the authors' list and must be a very rare
little volume, as, indeed, are all Johnson's botanical works, apart from the
immortal *Gerard em.*

In the second chapter, which forms the main bulk of the book, a chrono-
logical account of Johnson's life and work is given. With regard to the date of
Johnson's birth (which almost certainly took place at Selby in Yorkshire)
the authors are forced to admit that they have not advanced beyond the
statement made by Trimen and Dyer in 1869, that "it was probably at the
beginning of the seventeenth century." His early years are equally obscure,
and it is not until the first of his famous journeys (to Kent in 1629), when he
was an apothecary practising at Snow Hill, that we obtain any clear picture of
his activities. These journeys are here vividly described, and mention made
of many of the plants found by him and his fellow apothecaries. The authors
quote from the "somewhat free" account of the Kentish journeys which ap-
peared in *The Phytologist* for 1848. Those interested in the byways of Victorian
botanical literature might well read the whole of this article, and especially
the severe editorial censure called forth by certain passages, harmless enough
to our modern ears, on the refreshment taken by Johnson and his companions
during their journey (*The Phytologist*, 3, 125. 1848).

From this date (1629) until the beginning of the Civil War, Johnson led
an exceedingly active life, combining his practice as an apothecary with further
botanical excursions, and the publication of those works on which his fame
rests. In the Civil War he fought for the King and was mortally wounded in
1644 at the siege of Basing House, in which he distinguished himself by con-
spicuous bravery and of which the authors give an absorbing account com-
piled from contemporary reports.

In the third chapter Johnson's place in the investigation of the British
Flora is reviewed and it is here that the authors make their most important
contribution to botanical history. Hitherto it has been generally accepted
that the first British Flora was How's *Phytologia Britannica*, published in 1650.
It is pointed out, however, that Johnson's *Mercurius Botanicus* (published in
two parts, 1634 and 1641) contains not only a list of the plants found by him
on his journeys in the West of England, but also an enumeration of all the
then known indigenous British plants, and that it should therefore displace

the *Phytologia*, which was largely compiled from it, as the first British Flora. The fact that its claim has been so long overlooked is probably due to its rarity, and subsequent historians have relied on the statement by Pulteney (who only saw one part of the *Mercurius*) that How's was the first Flora of Britain.

The final chapter discusses the various genera which have been named *Johnsonia* in honour of the subject of this biography.

The book is illustrated throughout by drawings and facsimile pages from Johnson's works and deserves to be widely read by all interested in the history of British Field Botany.

J. S. L. G.

The Mechanism of Creative Evolution. By C. C. HURST, Ph.D., F.L.S. Pp. xxi + 364, with a frontispiece and 199 figures in the text. Cambridge University Press, 1932. 21s.

Dr Hurst's book gives an account of evolution regarded from a cytological point of view. It is concerned entirely with the genetic mechanism and makes no attempt to deal with the relations between the organism and its environment, either with regard to the possible origin of mutations or to subsequent selective processes.

The first chapters deal with the original genetical and cytological discoveries which were the starting points of the modern sciences of genetics and cytology. The author then proceeds to enumerate in considerable detail and with numerous examples the various cytological and genetical mechanisms which have been established up to the present time as playing integral parts in evolutionary change.

The book is confident in tone, perhaps too much so, in that the difficulties yet remaining are almost ignored. One example is the treatment of the origin of dominance, for though the author in his chapter on "Genes and Characters" when considering the genetics of rabbits and of man assumes that dominance is prevalent in the wild species, no attempt is made to account for this either on Fisher's theory of dominance or on any other theory. The possibility of the existence of further mechanisms and the limitations of existing theory are not discussed and it is difficult at times for the reader to distinguish between established fact and unproved theory.

In particular the concept of "genetical species" is open to criticism. Emphasis is rightly laid on the contribution that genetics and cytology can make to taxonomy, in relation particularly to the species concept: the statement is made that "On this view a species is no longer an arbitrary conception convenient to the systematist, a mere new name or label, but rather a real specific entity which can be experimentally demonstrated genetically and cytologically." This treatment of a species as a genetical entity divorced from its environment is as pernicious as the practice of regarding a species as a convenient herbarium unit equally divorced from its environment.

The question as to whether "species" exist would seem to depend primarily on two things (apart from such species as have arisen through polyploidy and hybridisation), viz. the size of mutations and the time during which mutations have been taking place in any group. For if it is believed that evolution occurs mainly by mutations of a single character and such mutations are taking place with any considerable frequency in all organisms all the time, the stability of species in nature will depend on elimination from among such mutations and this must in turn depend on environmental conditions and geographical isolation, in which case the concept of the "genetical species" can have little relation to actual fact. If, on the other hand, such mutations occur with considerable frequency only at considerable intervals of time, and if evolution takes place during such periods with subsequent stabilisation of species by the environment (and on the evidence at present before us, one would judge this to be the case) then the species is an entity determined in part by inheritable

differences (e.g. sterility, time of flowering) and in part by geographical and ecological barriers. Nevertheless it must in the first place have been determined by the environment. It is only on the third hypothesis, that evolution has taken place mainly by large mutations comparable in size to the differences between species, that the "genetical species" has much significance. This would seem improbable, at least in the case of animals and dioecious plants, as each such case would necessitate the simultaneous occurrence of two corresponding mutations. Finally it is probable that the status and character of "species" are basically different in different groups, or even within the same group (e.g. Dr Hurst's own group *Rosa*).

Apart from criticisms such as these, chapters IV–XIV are very valuable in their clear and concise description of the various methods of evolution and are particularly to be commended for their arrangement and the numerous illustrations which serve as examples and in many cases amplify the text.

The last chapters consist of speculations as to the origin of life and the course of its evolution, putting forward the idea that the first living organism was a free gene. As such they afford interesting and stimulating reading, although they are for the present pure speculation.

The book is written throughout in a clear and readable, though at times rather sensational style. It should be intelligible to the non-scientific reader as well as to the biologist and should give university students a good introduction to the subject. The book is throughout admirably and profusely illustrated. There is a bibliography which may be specially commended for its conciseness, and a good index.

E. F. W.

Plants: What they are and what they do. By A. C. SEWARD. Cambridge University Press, 1932. Pp. x + 141. 4s. 6d. net.

In the preface of this little book Professor Seward states that his aim is "to present a few aspects of plant-life, in language as free as possible from technical terms, to readers who have little or no knowledge of botany or other branches of natural science." The scope and plan of the book are admirable. The first four chapters deal with the comparison of plants with animals, the response of plants to stimuli, and the energy relations of plants; the next three with tissue differentiation and the green leaf; the eighth with roots; the ninth with bacteria and the nitrogen cycle; the tenth with seeds and seedlings, and the last two with the evolution of plants. There follow a useful list of elementary text-books and floras, a glossary and an index. The treatment is primarily functional, and care is taken to explain the chemical and physical terms which have been introduced.

Professor Seward has gone very far towards fulfilling his purpose. There are, however, occasional lapses from simplicity which must be stumbling-blocks to the non-scientific reader. Thus the explanation of the term "potential energy" on p. 23 is hardly adequate; and a statement of the wave theory of light (pp. 59–61) might well have been omitted in a book such as this (the behaviour of the leaf-pigments could have been described satisfactorily in terms of light of different colours).

A more serious objection is that the author, although explicitly avoiding a teleological expression of the behaviour of the root, has nevertheless made statements later which will almost certainly be interpreted teleologically by non-scientific readers, even if no botanist is misled. Thus, on p. 57, dealing with stomata, he writes: "the degree to which these are open or closed is under the control of the living protoplasm." This is the more open to objection because belief in a close stomatal regulation of transpiration is widespread though far from verified experimentally.

These, however, are small blemishes in a book which will be welcomed by all who desire "to appreciate what the world owes to plants."

A. R. C.

A NEW SPECIES OF *DRAPARNALDIOPSIS* (*DRAPARNALDIOPSIS INDICA* SP.NOV.)

·BY YAJNAVALKYA BHARADWAJA

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(With Plate VIII and 2 figures in the text)

THE material upon which the following communication is based was found on the leaves of grasses and sedges growing in the shallower water of a pond at Benares, India, in August, 1931. It formed a thin gelatinous pale green covering from which numerous long mucilaginous threads projected freely into the surrounding water. These threads are from 800 to 1800 μ thick (average about 1 mm.).

The principal axes are differentiated into elongate cylindrical, sometimes barrel-shaped internodal cells and short discoidal barrel-shaped nodal cells which alternate with one another in a very regular fashion (Text-fig. 1 A; Pl. VIII, fig. 1). The internodal cells are usually about 2–3 times as long as the nodal cells which are always slightly broader than the former except when the internodal cells are barrel-shaped. The greater part of the main axis is of more or less uniform breadth, but the apex and base are attenuated. The basal cells taper gradually, the lowest being usually prolonged into a rhizoid (Text-figs. 1 C, and 2 A and B), whilst the others give rise to numerous rhizoidal branches which fix the plant firmly to the substratum. In some cases the branching of the rhizoids is so profuse that they form a dense cortex around the base of the main axis (Pl. VIII, fig. 3). There is practically no differentiation into nodal and internodal cells in the basal part of the main axis. Normally every nodal cell bears lateral branches and there are typically four of them, although they exhibit varied differentiation as the following account will show.

The main axis usually bears a number of lateral branches of un-
limited growth, repeating its structure and ultimately attaining the
same size. These branches arise from the median portion of some of
the nodal cells which may produce from one to four such branches
each.

Both the main axis and the laterals of unlimited growth bear
richly ramified short branches of limited growth, arising as opposite
pairs or in whorls of four from the median portion of the nodal cells.
The same nodal cell may bear both branches of unlimited and limited
growth. As an exception both kinds of branches may originate a
little above or below the middle of the nodal cell.

The laterals of limited growth are set at right angles to the main
axis and are broadly orbicular in outline owing to the spreading
character of their branches (Text-fig. 1 D). There is no evidence of a
main axis in these laterals. Their branches are subulate and the
distal ends terminate in long hyaline hairs consisting of one or more
greatly elongated cells in which the chloroplasts are disorganising
or have totally disappeared (Text-figs. 1 B and D, 2 D; Pl. VIII,
fig. 1). The basal cells of the laterals of limited growth bear from
two to six sub-apical branches and are cuneiform in shape with a
short cylindrical projection bearing each branch. The basal cell of
each such primary branch generally has a similar shape and in its
turn bears secondary branches at its upper end. All the cells of the
laterals of limited growth are much narrower than those of the main
axes. Similar short laterals may also be borne on some of the basal
cells of the main axis that bear rhizoids, but such laterals do not
always arise from the middle of the cell, nor do they always grow to
their full size (Text-fig. 2 A and B). The entire thallus is enveloped
in mucilage within which several blue-green algae were growing.

Growth is intercalary and new cells may be formed by division in
any of the axes of unlimited growth, whilst laterals of both limited
and unlimited growth may arise secondarily at any point in the
thallus. An internodal cell in a long axis may divide transversely
into two daughter cells which grow to the length of the mother cell.
The lower cell of the pair then divides into two, the lower half again
enlarging to the full size, whilst the upper remains small and con-
stitutes a new nodal cell between the two internodal ones. In this
way the characteristic alternation of internodal and nodal cells is
maintained. In rare cases there is only one division and, of the two
resulting cells, the lower develops into an internodal while the upper
forms a nodal cell, so that two nodal cells come to lie adjacent to one
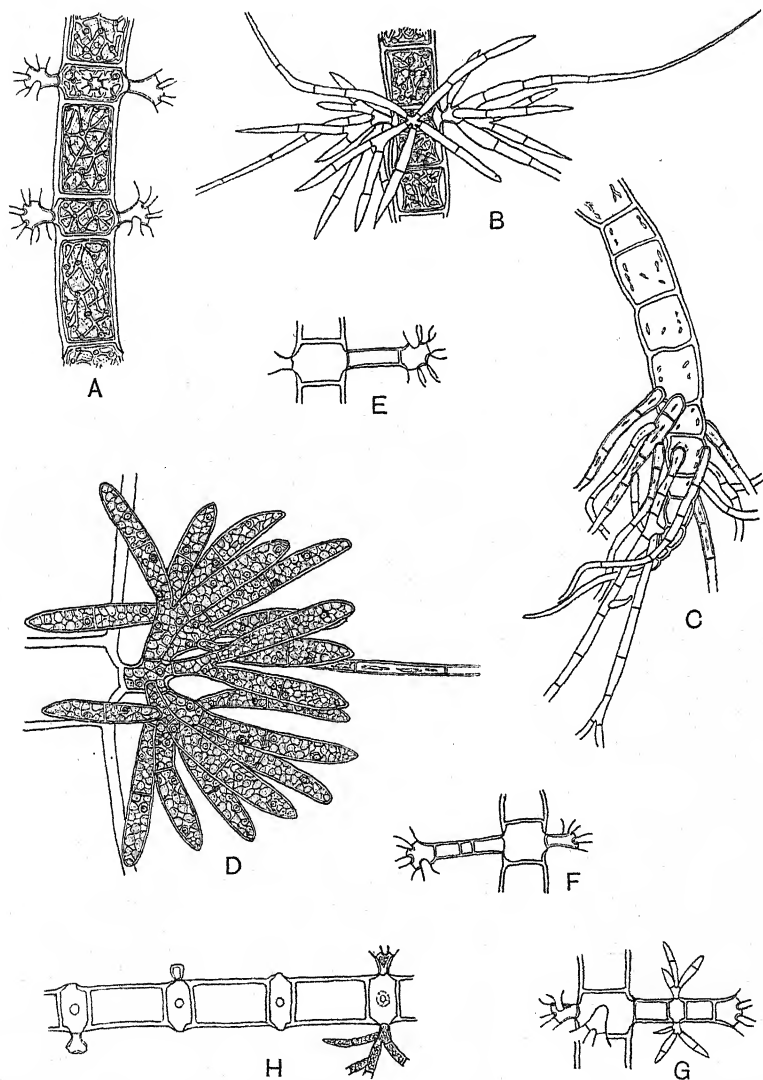
Text-fig. 1. *Draparnaldiopsis indica* sp.nov. A, portion of main axis with internodal and nodal cells showing chloroplasts. B, the same with short laterals. C, basal portion of axis with rhizoids. D, part of a nodal cell bearing a short lateral. E, F and G, stages in the development of a long lateral from one of limited growth. H, portion of main axis from which the short laterals have dropped off, leaving only scars or remains of branches on the nodal cells. (A–C and E–H × 220; D × 485.)

another. Sooner or later such nodal cells give rise to one or more laterals of limited growth. The first step in their formation is the development from the middle of the cell of a small cylindrical protuberance which subsequently becomes separated by a transverse wall and constitutes the first cell which then divides to form a row of two or three cells. At this stage the basal cell begins to form the primary branches. These first appear as short papillae which are soon cut off by a transverse wall.

The maximum number of laterals borne on a nodal cell is four, all of which may be of unlimited or limited growth (Text-fig. 1 B) or the laterals may be partly short and partly long. Moreover, one finds many transitions between the two types of branches. Certain of the short laterals possess two basal cells, either similar or the lower much longer than the upper (Text-fig. 1 E) which bears normal branches; others have three, and still others four basal cells (Text-fig. 1 F) of which the uppermost bears branches and the middle one of the lower three is short and nodal in character. In some of the laterals of the last type this nodal cell bears short branches (Text-fig. 1 G). In the same way short laterals are to be found with three internodal and two nodal cells at their base, the nodal cells bearing short branches. Between such cases and the typical lateral of unlimited growth there are many other transitions. It thus appears probable that the laterals of unlimited growth arise by basal growth of certain of the laterals of limited growth and that the former are but a modification of the latter. The growth of a long branch is initiated by the division of the basal cell of the short lateral into two, of which the lower subsequently divides into three, after which further elongation is effected by intercalary growth, the apical portion of the long lateral thus arising still preserving the original character of the branch of limited growth.

The rhizoids produced from the basal cells of the main axis generally arise as independent structures (Text-fig. 1 C), though in some cases they probably represent modified short laterals (Text-fig. 2 A and B) (cf. below). In the former case they originate in the same way as the branches of limited growth, but they may grow out from any part of the basal cells which, as already stated, are not usually differentiated into internodal and nodal cells (Text-fig. 1 C). The initial cell of the rhizoid divides repeatedly to form a comparatively long thread before lateral branches are produced; these branches arise at any point on the cell. The cells of the rhizoids are elongate and their chloroplasts gradually disorganise and ultimately almost disappear.

Apart from those formed by the basal cells of the main axis, similar branched rhizoids may develop from any part of a lateral of limited growth (Text-fig. 2 C) or may replace the whole of such a lateral (Text-fig. 2 E, *l.*). Such rhizoidal development may occur on any node, but it is invariably found on nodes which bear branches of unlimited growth (Pl. VIII, fig. 2). Sometimes, similar rhizoids also originate from internodal cells immediately adjoining nodes bearing rhizoids. In the former case one or more branches of a short lateral may develop as a rhizoid (Text-fig. 2 A–D; Pl. VIII, fig. 4) and all transitions are found between a lateral with only one or two rhizoidal branches and a lateral completely replaced by a branched rhizoid (Text-fig. 2 E, *l.*). Occasionally one meets with a long rhizoidal filament bearing a few normal branches at its apex (Text-fig. 2 G, *r.f.*). Sometimes a nodal cell bears branched rhizoids only in place of all of its short laterals (Text-fig. 2 F). These facts indicate that the rhizoids of this alga are most probably to be regarded as homologous with laterals of limited growth the branches of which, by repeated division and elongation of their cells, have become transformed either wholly or in part into long branched threads. Thus laterals of limited and unlimited growth and rhizoids are all homologous structures.

Those rhizoids which originate from internodal cells, adjoining nodes bearing rhizoids, emerge in the neighbourhood of the septum (Text-fig. 2 D–F). Rhizoids originating on or in place of the laterals of limited growth on nodes bearing branches of unlimited growth develop only after the latter have reached a certain size; when the long laterals are young there is no trace of rhizoids. In rare cases rhizoids have also been observed to develop from the basal cells of the long laterals. The later development of rhizoids at these points may be so considerable owing to their profuse and dense branching that a thick cortical covering is formed around the axis, totally concealing both it and the bases of the branches of unlimited growth (Text-fig. 2 G; Pl. VIII, fig. 2). The rhizoids are sometimes so closely wound round the axis as to produce an appearance of compression, the cells at such points being much narrower than elsewhere. Such dense growths of rhizoids would appear to serve a mechanical function, no doubt materially strengthening the attachment of the freely projecting long laterals with the main axis. The rhizoids generally possess the same breadth as the cells of the branches of limited growth. Those produced on the basal cells of the main axis have slightly thicker walls than the others.
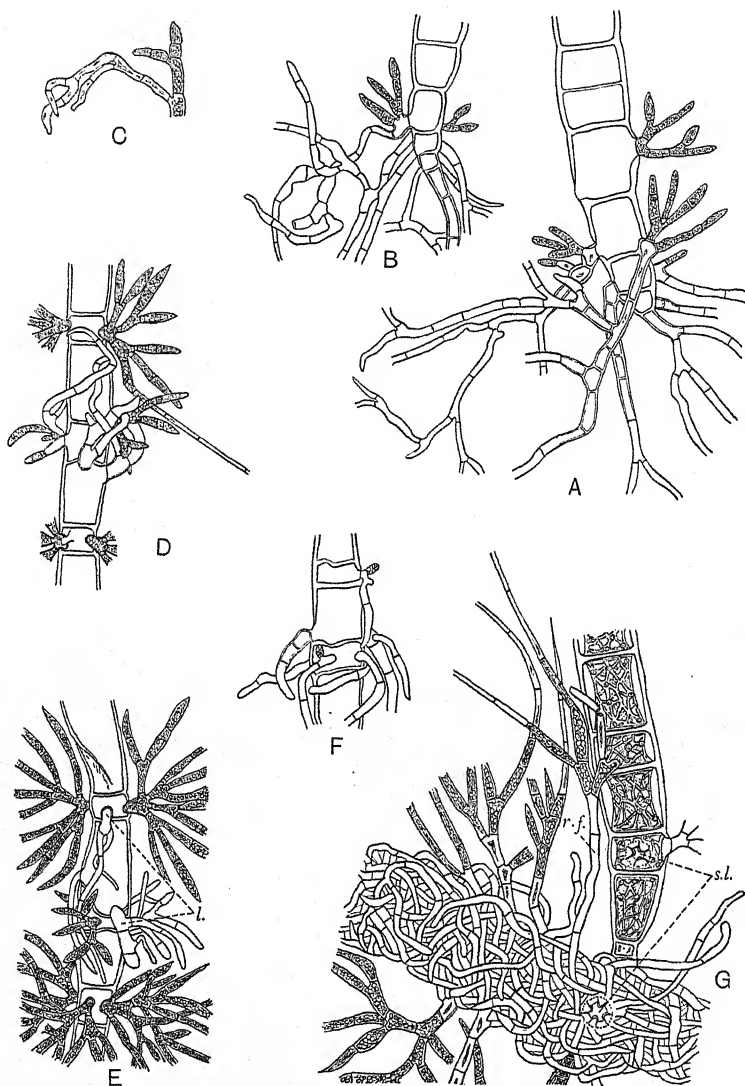
Text-fig. 2. *Draparnaldiopsis indica* sp.nov. A and B, basal portions of main axes showing rhizoids developed from portions of short laterals. C, short lateral with terminal cells transformed into a branched rhizoid. D–F, portions of axes bearing rhizoids on the nodal and internodal cells, the former appearing as partial or complete modifications of short laterals. G, portion of main axis concealed by a cortical covering of rhizoids formed near the place of origin of a long lateral. *l.* short lateral completely transformed into a branched rhizoid; *r.f.* rhizoidal filament bearing at its apex a few normal branches; *s.l.* base of short lateral not shown at its full length in the figure. (All × 220.)

Each cell of the long axes has a parietal chloroplast in the shape of a reticulate cylinder with entire margins and several pyrenoids, occupying the whole length of the cell (Text-figs. 1 A and B, and 2 G). In the cells of the branches of limited growth the chloroplasts are likewise parietal and reticulate, with one or sometimes two pyrenoids, and densely fill the cell (Text-fig. 1 D).

Zoospore or gamete formation has not been observed, but in material of old filaments, that had not been teased out, many of the nodal cells lacked branches of limited growth or only bore remains of such branches, sometimes merely the cuneiform basal cells (Text-fig. 1 H). This possibly implies that the branches of limited growth break away from the long axes either entirely or in part during the process of zoospore or gamete liberation.

In 1929 Smith and Klyver[3] published an account of an alga resembling that described above in most essential respects and created for it a new genus *Draparnaldiopsis* with the single species *D. alpinis*. The Indian alga agrees with this form in the following characters: (i) the alternation of long internodal and short nodal cells in the main axis and the other branches of unlimited growth, (ii) the origin of the branches of limited growth from the median portion of the nodal cells only, (iii) the cuneiform shape of the basal cells of the branches of limited growth and the fact that they always produce a number of branches near their apices, the basal cells of which branch in the same manner, (iv) the termination of these branches in long hairs consisting of one or more cells without chloroplasts, and (v) the envelopment of the thallus within a hyaline mucilaginous envelope. There are, however, some significant differences, viz. (*a*) the main axis usually bears a number of branches of unlimited growth which arise from the nodal cells and replace some of the short laterals; (*b*) the branches of limited growth arise not only in pairs but also usually in whorls of four and the basal cell of each bears 2–6 branches; (*c*) the chloroplasts, both in the cells of the main·axis and in those of the branches of unlimited growth, are cylindrical and reticulate and do not constitute merely equatorial girdles, as in *D. alpinis*; (*d*) the chloroplasts in the cells of the branches of limited growth are of the same pattern and completely fill the cells, while Smith and Klyver describe the chloroplasts in similar cells in the American form as laminate and parietal, though the exact form of the chloroplast is not clear from their figures; (*e*) the attaching rhizoidal system is much more strongly developed; (*f*) rhizoids may develop on any of the branches of the laterals of limited growth or may altogether

replace them; (*g*) in rare cases rhizoids may also originate from internodal cells; and (*h*) the growth of rhizoids is especially profuse at the points of origin of the branches of unlimited growth where they form a cortical covering around the axis and the bases of the branches.

Minor points of difference lie in the character of the plant mass with its long projecting mucilaginous threads and in the occasional barrel-shaped cells of the long axes. The rhizoids at the base of the main axis are branched and sometimes appear to represent modifications of the branches of limited growth. The rhizoids borne elsewhere appear always to be more or less complete modifications of the branches of limited growth. The cells of the main axes and their long branches are larger than those in the American form, while the branches of limited growth (excluding the terminal hairs) are not so long. The habitat of the Indian alga is quite different since it occurs practically at sea-level.

The general character of the plant-body in both forms is quite like that of a *Draparnaldia* in which likewise the basal portion of the main axis is attenuated and bears rhizoids (cf. Berthold (1), Taf. 3, Fig. 4). The chloroplasts in the cells of the branches of unlimited growth in the Indian alga are like those of *D. platyzonata* Hazen (cf. Hazen (2), Pl. 41), although in other species of *Draparnaldia* they are equatorial and girdle-shaped. The production of rhizoids at the points of formation of branches of unlimited growth has been described by Berthold (Berthold (1), Taf. 3, Fig. 2) in *D. glomerata* (Vauch.) Agardh., where, however, they are not produced in anything like the same profusion and do not form a cortical covering.

The American and the Indian algae thus show a great measure of general resemblance with *Draparnaldia*, and the only significant differences are the alternation of long internodal and short nodal cells in the main axis and its branches of unlimited growth and the restriction of the branches of limited growth to the nodal cells. Moreover, the growth of the thallus in these forms, as at present observed, is more markedly intercalary than in *Draparnaldia* (cf. Berthold (1), p. 204), though the absence of apical growth of the type described by Berthold for the latter genus cannot be positively dismissed, since there has been no opportunity of examining growing material of the Indian form. The account of the American species also leaves open the possibility of the growth not being entirely intercalary. A minor point of difference from *Draparnaldia* lies in the shape of the basal cells of the branches of limited growth and in

their mode of branching. These differences, which appear to be shared both by the American and the Indian algae, are probably sufficient to warrant the retention of the genus *Draparnaldiopsis*.

The Indian form described here is clearly a new species of the genus whose diagnostic characters are slightly modified in order to include both the American and the Indian species which may be named *Draparnaldiopsis indica*.

Draparnaldiopsis Smith and Klyver, diagn. emend.

Thallus macroscopic, filamentous, differentiated into long and short branches, the former composed of alternating long internodal and short nodal cells; branches of both kinds formed only on the short cells and arising from their median region to the number of 1–4. Cells of the short branches much smaller than those of the long ones, the basal cells cuneiform and bearing up to six branches at their apices where basal cells branch in the same way. Branches of the short laterals terminating in long hairs. Chloroplasts single, girdle-shaped and reticulate or laminate with from one to several pyrenoids. Rhizoids confined to the base of the plant or, in one species, arising abundantly on nodal cells, especially those bearing long branches as more or less complete modifications of the short branches, sometimes forming a dense cortical covering around the main axis and the bases of the branches. Thallus invested with a copious hyaline gelatinous envelope, attached to the substratum by the basal rhizoids. Reproduction unknown.

(1) *D. alpinis* Smith and Klyver.

Trans. Amer. Microsc. Soc. **48**, 1929, p. 200.

(2) *D. indica* sp.nov.

Cells of branches of unlimited growth cylindrical or barrel-shaped; basal cells of laterals of limited growth bearing two to six branches. Lateral branches solitary, opposite or in whorls of four, those at a node all of limited or all of unlimited growth or some of the one and some of the other; chloroplasts (except in cells of hairs and rhizoids) parietal and reticulate, occupying the whole length of the cell. Rhizoids copiously developed at the base of the plant as well as on the nodes of the branches of unlimited growth, especially when these produce similar branches. Rhizoids with numerous branches.

Internodal cells 28–63 μ (average about 35 μ) long and 20–60 μ (average about 30 μ) broad; nodal cells 8–31 μ (average about 16 μ)

long and 16–60 μ (average about 33 μ) broad; cells of short laterals 4·2–8·4 μ broad; width of the thallus including the hairs on the short laterals 800–1800 μ (average about 1 mm.), without the hairs 160–500 μ (average about 250 μ).

In a pond in Benares, India, August, 1931.

The author wishes, in conclusion, to express his great indebtedness to Prof. F. E. Fritsch, F.R.S., for his guidance and criticism.

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EXPLANATION OF PLATE VIII

Draparnaldiopsis indica sp.nov.

Fig. 1. General habit. (×73.)

Fig. 2. Main axis totally concealed by a cortical covering of rhizoids around the place of origin of a long lateral. (×160.)

Fig. 3. Basal portion of main axis, totally concealed by a covering of rhizoids. (×90.)

Fig. 4. Portion of axis with partial transformation of a short lateral into a branched rhizoid. (×300.)

Note. After sending this communication to press a paper by Meyer (*Rev. Alg.* **2**, 241 *et seq.*, 1925) came to my notice dealing with a number of new species of *Draparnaldia*. In one of these (*D. baicalensis*) Meyer describes the formation of a dense cortical covering around the axis due to a profuse growth of rhizoids similar to that described in this paper.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

BHARADWAJA—A NEW SPECIES OF *DRAPARNALDIOPSIS*
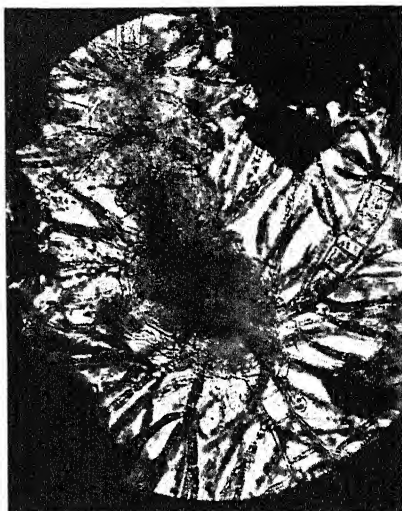
SPORE DISCHARGE IN THE ASCOMYCETES

I. PYRENOMYCETES

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(With 10 figures in the text)

CONTENTS

I. TYPES OF SPORE DISCHARGE

THE object of this paper is to give a comprehensive account of the spore discharge in Pyrenomycetes[1]. It must be recognised at the outset that discharge is only the preliminary step in spore dispersal. The function of this discharge is to free the spores, which are invariably sticky, from immediate contact with the parent body so that they may subsequently be dispersed. In the majority of cases dispersal is brought about by air currents, but in the coprophilous forms animals are the agents concerned.

The ascus, in Pyrenomycetes, is essentially an explosive sporangium except in certain clearly degenerate forms (e.g. *Chaetomium*). It is usually elongated and limited by a thin elastic cell wall. Within this, in the mature ascus, is a very thin lining layer of cytoplasm, the semi-permeable membrane of the ascus. This encloses a relatively large volume of ascus sap, which is probably an aqueous solution of sugars derived from the glycogen which is present in the epiplasm of the young ascus. The spores hang together more or less firmly by their own stickiness and are suspended in the solution in the upper region of the ascus by a mucilaginous strand attaching the apical spore to the tip of the ascus. When mature the ascus is in a state of strain, the elastic wall being greatly stretched by the osmotic pressure of the ascus sap. Finally rupture occurs, by the opening of a pore in an apical region of extra thinness (e.g. *Leptosphaeria*), by the dislodgment of an apical plug (e.g. *Hypocopra*) or by the separation of a distinct cap (e.g. *Podospora*). Rupture is followed instantly by the

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[1] In this account the *Erysiphales* are not considered.

contraction of the elastic wall, which squirts out the ascus contents to a distance ranging from a few millimetres in most cases up to nearly half a metre in *Podospora curvicolla*.

In Pyrenomycetes the tip of the ascus must reach the ostiole before explosion occurs. Owing to the narrowness of the canal leading from the cavity of the perithecium to the outside, only one ascus can, as a rule, reach the ostiole at a time, so that simultaneous discharge of asci is generally impossible.

There has been little careful work on the question of spore discharge in the Pyrenomycetes, but so far as observations have been made four distinct methods of spore discharge can be recognised which will now be considered in turn.

Type I. Attached ascus with single thin elastic wall

This is probably the commonest and most primitive type from which all the others have been derived. It has been described and figured by numerous workers including Zopf[14], Griffiths[5], Buller[2] and Ingold[7].

As an example of a fungus belonging to this type we may take *Podospora curvula*, a form which very frequently appears on horse-dung and which is particularly favourable for study on account of the semi-transparent nature of the perithecium wall (Fig. 1).

The asci and paraphyses occur at the bottom of the perithecium attached to a basal cushion of pseudoparenchymatous tissue. These asci are at all stages in development. In *P. curvula* the mature ascus is elongated and very much swollen but it always remains attached to the basal cushion. In a perithecium actively engaged in spore discharge the lower region is full of immature and semi-mature asci, while the upper region is occupied by the swollen upper parts of the fully mature ones. The top part of the perithecial cavity tapers and finally merges into the narrow canal which leads to the outside. If a perithecium is carefully removed from its substratum and mounted in a drop of water on a glass slide the whole process of ascus growth and spore discharge may be observed. The tip of a single one of the mature asci in the upper region of the perithecium may be seen, through the transparent wall of the perithecium, to be slightly in advance of the others. If this is carefully watched it will be seen to elongate, perceptibly growing up the narrow canal of the perithecium neck. Before the tip reaches the opaque region of the neck it becomes tightly fitted in the canal, which is lined with numerous upward projecting periphyses against which the ascus wall presses. Finally
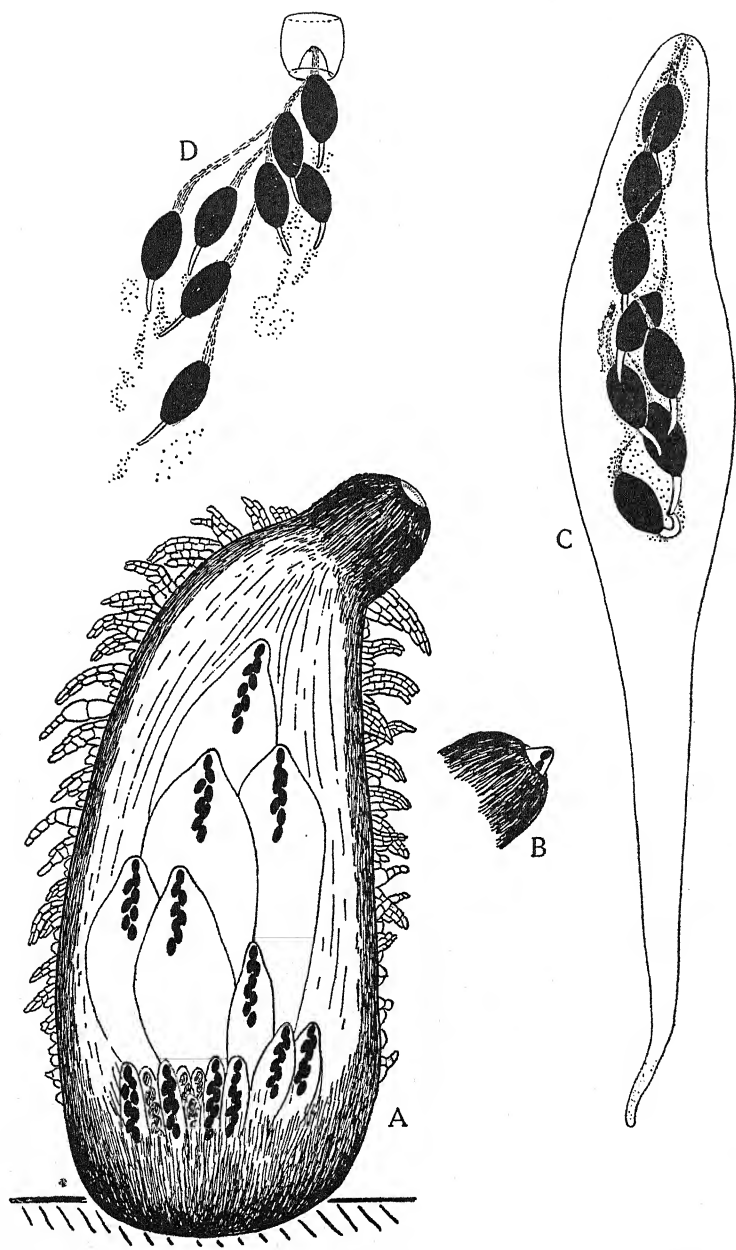
Fig. 1. *Podospora curvula.* A, perithecium with asci showing through the transparent wall, ×104. B, ostiole region of the same perithecium 10 min. later; the tip of the ascus which was leading in A has now reached the outside and is about to discharge its spore mass, ×104. C, a single ascus which has just commenced to swell, ×335. D, a discharged spore mass showing the ascus cap attached to the apical spore, ×335.

the tip of the ascus reaches the ostiole and protrudes slightly beyond it (Fig. 1 B). Then the ascus explodes and ejects its contents together with its cap (Fig. 1 D). In the particular case shown in Fig. 1 10 min. elapsed between the stage shown in A and that shown in B, where the ascus tip is just projecting beyond the ostiole. A second later, without any further elongation, the ascus burst.

As a result of discharge the elastic wall of the ascus immediately contracts, and being still attached to the basal cushion it is drawn back into the bottom of the perithecium as a shrivelled empty envelope which probably soon disintegrates. Immediately one ascus has discharged its contents another one begins to elongate up the neck, and so the process goes on in orderly succession.

The essential features of this type of discharge are firstly that the ascus always remains attached to the base of the perithecium, and secondly that the ascus reaches the ostiole by elongation involving the gradual stretching of the ascus wall.

Type II. Attached ascus with double wall

This type is probably fairly frequent in the Pyrenomycetes and represents a highly evolved and specialised form. It has been noted and figured by Pringsheim (on *Pleospora*(9)), Hodgetts (on *Leptosphaeria*(6)), Griffiths (on *Sporormia*(5)), and the writer has observed it also in the case of *Sporormia* and *Melanomma*. Hodgetts' account is particularly clear and beautifully illustrated. As an example we shall consider *Sporormia intermedia* (Fig. 2), a very frequent member of the dung flora. As in the previous type, the asci occur in the perithecium attached to the base and intermixed with paraphyses. The chief feature of this type is that the ascus has a "jack-in-the-box" structure. The ascus wall consists of two layers: (1) an outer relatively thick rigid membrane, and (2) an inner very thin elastic membrane. When ready for discharge the apex of the rigid membrane is ruptured and the inner elastic membrane then elongates, at first almost explosively, up the neck of the perithecium. In the case of *Sporormia* the ascus may project considerably beyond the ostiole before it explodes to liberate, simultaneously, the enclosed spores. *Leptosphaeria arcuta* and *Pleospora scirpi* exhibit certain slight differences from *Sporormia*. In these two fungi the spores (as in the case of *Geoglossum* in the Discomycetes) are discharged successively from the ascus through an apical pore. As soon as this aperture is formed a spore is pushed into it which blocks the ascus for a second, then gathers
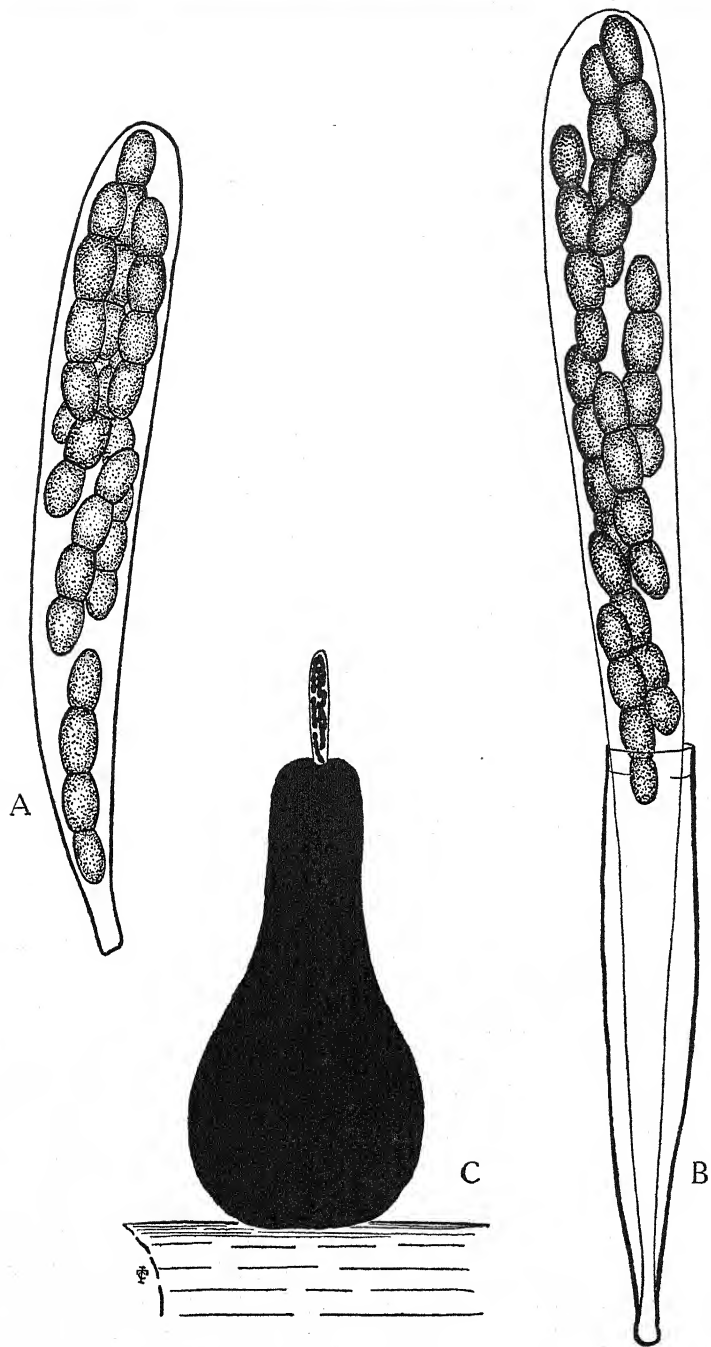
Fig. 2. *Sporormia intermedia*. A, ascus before rupture of the outer wall, ×842. B, another ascus after rupture of the outer wall and elongation of the inner wall, ×842. C, perithecium, on piece of straw in horse-dung, showing protruding ascus tip, ×190.

velocity and is discharged. Instantly its place is taken by another spore and so on until the whole eight are ejected. This successive type of discharge is not fundamentally different from simultaneous discharge and has probably been evolved, in connection with elongated spores, along several lines of evolution in Ascomycetes. Indeed simultaneous discharge is, as pointed out by Falck (4), probably to be looked upon as a successive type. The interval between the discharge of spores being so slight as to be unappreciable, discharge of spores appears to be simultaneous. When dispersal by air currents is the rule this isolation of the individual spores of an ascus is extremely important, since without some mechanism for separating the spores they would naturally hang together in a mass which would too rapidly fall to the ground. The whole question of the separation of the spores of an ascus from one another is discussed in full by Buller (1). It might be mentioned that in most cases the diameter of the ascus opening is less than the diameter of the spore, hence successive discharge is almost inevitable.

The "jack-in-the-box" type represents a mechanism for rapid ejection of spores under suitable conditions. The rigid outer ascus wall allows the ascus to store its pressure within a confined space, ready to spring up to the ostiole when conditions (e.g. water supply) are favourable.

Type III. Long-necked form containing detached asci

This type is highly evolved and probably derived originally from the primitive type (type I). Although it has only been observed in a few cases there seems every reason to believe that it will prove to be the general method of spore discharge in the long-necked members of the Pyrenomycetes. It has been observed in *Endothia parasitica* by Rankin (10), in *Gnomonia rubi* by Dowson (3) and by the writer in *Ceratostomella ampullasca*. *C. ampullasca* occurs on the rotting wood of oak. The spherical perithecium is immersed in the substratum and from it a long narrow neck passes to the outside. The following description is based on observations made on perithecia mounted in water. Within the perithecium the asci arise from a basal cushion of tissue from which they are budded off when mature. There are few paraphyses. The cavity of the flask becomes completely filled with a mass, innumerable thousands, of small asci (Fig. 3 B) most of which are detached. Although the asci are detached they are not arranged in haphazard fashion, since their mutual pressure maintains them in an erect position with the apices pointing towards the neck

canal. Due to the pressure produced by the developing asci, the mature ones are squeezed out of the perithecium, passing in single file up the narrow canal of the neck. In the case of *C. ampullasca* the neck is opaque and the actual movement of the asci in the canal cannot be observed. The neck opens by a narrow slit-like ostiole. Arrived at this ostiole the ascus pushes open the slit and protrudes until the spore-containing part of the ascus is exposed to view (Fig. 3 A). The lower part of the ascus is still firmly held by the ostiole. At this stage the ascus bursts, discharging its spores. Immediately after rupture the empty ascus envelope is pushed out by the ascus below, and so on. This is an exceedingly quick-firing type of discharge, only a few seconds intervening between successive ejections. Observations in air show that the spores are shot to a height of several millimetres and that the rate of discharge is just as rapid as in the case of perithecia discharging in water.

Dowson's account of *Gnomonia rubi* agrees precisely with the present writer's observations on *Ceratostomella*. However, in *G. rubi* Dowson was actually able to see the single file of asci passing up the perithecium neck.

Rankin's observations on spore discharge in *Endothia parasitica* are of interest because they represent the first description of this type of discharge, and also because they indicate certain slight departures from the *Ceratostomella* type. In *Endothia*, according to Rankin, a film of water covering the ostiole is necessary for discharge. In the case of *Ceratostomella* such an elastic film would undoubtedly interfere with, and probably entirely prevent, the discharge of ascospores. Again in *Endothia*, as opposed to both *Ceratostomella* and *Gnomonia*, the ascus appears to pass completely beyond the limits of the neck before it explodes. The method of spore discharge in *Endothia* can best be explained in the words of Rankin: "The asci are formed at the base of the perithecium and are then detached as others form. The cavity is thus filled with detached asci. The asci which usually fill the perithecium are pushed up through the neck due largely to the swelling of the asci themselves. If free water is present on the surface of the stroma, even in so slight a quantity as a drop at the ostiole, spores are forcibly ejected into the air by the rupture of the ascus at the surface of the film of water. The dehiscence may be observed with a hand lens or the 4 or 16 mm. objectives. In either case the only visible phenomenon is the sudden and regular breaking of the film of water over the ostiole. This gives the impression for the moment of a point of light."
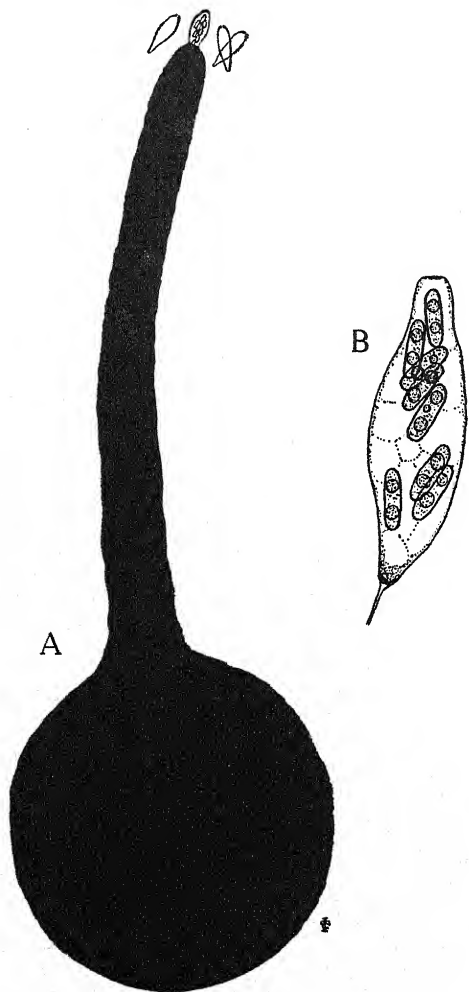
Fig. 3. *Ceratostomella ampullasca*. A, a perithecium discharging. An ascus
still held in the ostiole is just about to explode. Three empty ascus
envelopes are to be seen not far from the ostiole. The three spore groups
ejected by these asci are visible some way in front of the ostiole, ×80.
B, a single mature detached ascus, ×617.

The detached ascus type represents one in which a rapid method of spore discharge has been evolved which can take place in a long-necked perithecium. The three genera *Endothia, Gnomonia* and *Ceratostomella* all agree in having long necks and comparatively small asci. It is clear that had an attached ascus to elongate from the basal cushion up through a neck canal 1 or 2 mm. in length, spore discharge would necessarily be slow. This consideration leads us to suggest that the detached ascus type of violent discharge is the normal type of discharge in the long-necked Pyrenomycetes.

Type IV. *The non-explosive type*

In a number of degenerate forms the asci have lost their explosive nature. Thus in *Chaetomium* the wall of the ascus when mature deliquesces and the perithecium becomes filled with a mass of spores embedded in mucilage. Under suitable conditions this mucilage absorbs water and the spore mass exudes through the ostiole, like paste squeezed out of a tube, to form a spore tentacle. This type of discharge may occasionally occur in forms which normally have explosive asci.

II. The distance to which spores are shot

The height to which a spore or spore mass is discharged depends on two important factors, namely the initial velocity given to it by the discharging ascus and the mass of the projectile. In the case of a minute sphere projected upwards the height attained is determined by the initial velocity of the sphere, its mass and the viscosity of the air. The effect of gravity can be neglected.

The initial velocity, U, is given approximately by[1]

$$U = kX,$$

where X = the height to which the spore mass rises, k = a constant. This constant is given by

$$k = \frac{6\pi\mu r}{m},$$

where μ = the viscosity of the air, r = the radius of the sphere, and m = the mass of the sphere.

From this equation it is clear that with a given initial velocity of discharge increase in the diameter of the sphere gives an increase in

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[1] The writer has to thank Dr W. N. Bond for deducing this formula. It is exactly the same as that given by Buller(1) for the horizontal projection of basidiospores.

the height to which it is projected[1]. This explains how it is that such a large mass as the projectile of *Podospora fimiseda* is shot to a much greater distance than a small body such as a single spore of *Hypoxylon coccineum* (Fig. 4).

PODOSPORA FIMISEDA.

0·1 mm.
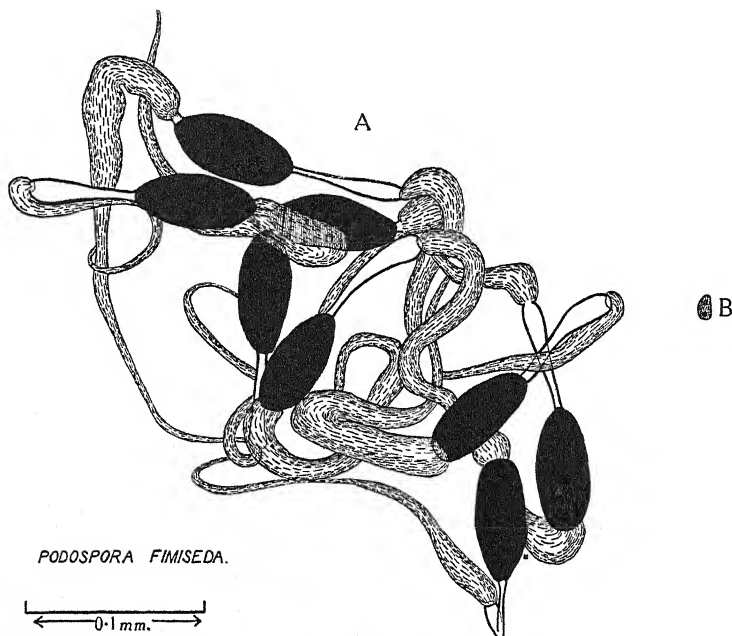
Fig. 4. A, spore mass of *Podospora fimiseda*. The large spores are held together by strands of mucilage exuded by the apex of the spore and by the appendage cell. B, single spore of *Hypoxylon coccineum*. Both drawn to the same scale.

Table I gives the distance of discharge of some spores and spore masses of the Pyrenomycetes.

The most striking feature illustrated by this table is the great difference in distance of discharge between those forms in which

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[1] A sphere of radius $3\cdot35\,\mu$ corresponds in volume with a spore of *Hypoxylon coccineum* ($12 \times 5\,\mu$). The spore mass of *Podospora fimiseda* may be estimated as being at least twice the volume of the ellipsoidal spores in it. By multiplying the volume of a single spore ($60 \times 30\,\mu$) by 16 we get a very rough idea of the size of the projectile. This corresponds to a sphere having a radius of $47\cdot6\,\mu$. Assuming that the density of the spores is 1 (actually it is probably slightly greater than this) we find by substitution in the formula given above, that for a sphere of $3\cdot4\,\mu$ radius to be shot $0\cdot7$ cm. (the height to which the spores of *H. coccineum* are projected) an initial velocity of 5270 cm. per sec. is necessary, while for a sphere of radius $47\cdot6\,\mu$ to be projected 30 cm. an initial velocity of only 1120 cm. per sec. is required.

single spores separate out and those which discharge spore masses. In all cases so far described the "spore mass" associated with long-distance discharge is a feature of certain specialised members of the coprophilous flora[1]. In fact, the evolution of the spore mass is not only found amongst certain specialised Pyrenomycetes, but also in

TABLE I

Species	Spore dimensions* Length μ	Spore dimensions* Width μ	Notes	Maximum distance of discharge in mm. Horizontal	Maximum distance of discharge in mm. Vertical	Observer
Leptosphaeria acuta	36–50	5–6	Spores discharged successively	20	12	Hodgetts (6)
Hypoxylon coccineum	10–12	4–5	Spores not forming a spore mass	7·5	—	C.T.I.
Hypomyces lactifluorum	18–20	4–4·5	Spores not forming a spore mass	10	10	Buller (2)
Podospora fimiseda	50–60	28–30	8 spores form a mass	—	300	C.T.I.
P. curvula	22–30	14–15	8 spores form a mass	—	200	C.T.I.
P. curvicolla	14	8	128 spores form a mass	—	450	Weimer (11)
Sordaria fimicola	19–22	10–12	8 spores form a mass	—	60	Griffiths (5)

* From Winter (12).

some coprophilous forms, also specialised, which belong to other groups of fungi. It is of great interest to compare the biology of spore discharge in three such widely separated fungi as *Pilobolus*, *Ascobolus immersus*, and *Podospora*. In all three cases discharge takes place towards the light, the direction being governed by means of a heliotropic mechanism situated, in *Pilobolus*, in the sporangiophore, in *Ascobolus* in the individual projecting asci, and in *Podospora* in the perithecium neck. A spore mass is involved in each case, with the spores held together in a mass of mucilage, which also seems to cement the discharged masses to the herbage surrounding the dung. All these spore masses can be shot to a relatively great distance, e.g. 20 cm. in *Podospora curvula*, 30 cm. in *Ascobolus immersus*, and 200 cm. in *Pilobolus longipes*. In each case discharge occurs during the day, and the spore protoplasm is protected from the injurious effect of light. This protection takes the form of a dark spore coat

[1] Massee drew attention to this fact. He pointed out that in *Xylaria* a spore mass in which the eight spores are cemented together in mucilage is only developed in the two coprophilous species of the genus.

in *Podospora* and *Ascobolus*, and of a special black cap in *Pilobolus* which effectively shades the pale or colourless spores from light rays. The accompanying diagram (Fig. 5) brings out the chief features
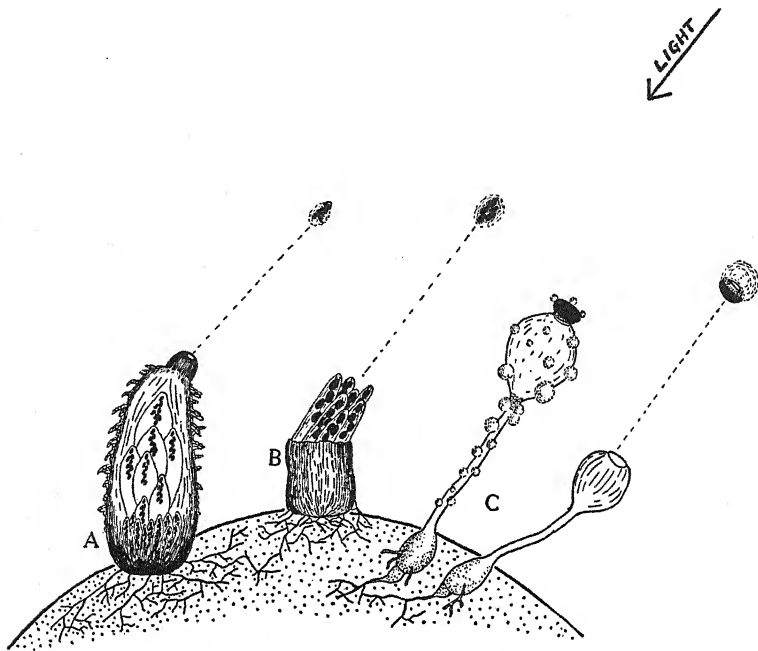
Fig. 5. Diagram to illustrate the parallel evolution in the three forms A, *Podospora curvula*, B, *Ascobolus immersus* and C, *Pilobolus Kleinii*. These are not drawn to the same scale. The substratum of dung is indicated by stippling.

which indicate the parallel evolution of these three remarkable coprophilous forms.

III. PERIODICITY OF SPORE EJECTION

It is of interest to consider how far spore discharge in the Pyreno-mycetes is a steady process and how far it tends to fluctuate in rate. Work on this question has shown, in every form investigated, clear evidence of a diurnal periodicity of spore output. A simple method for this investigation was described by the writer (7). In more recent work it has been found convenient to use an apparatus which will give a continuous record over a period of 24 hours. This is shown in Fig. 6 which gives full details of the construction. *A* is a glass frame-
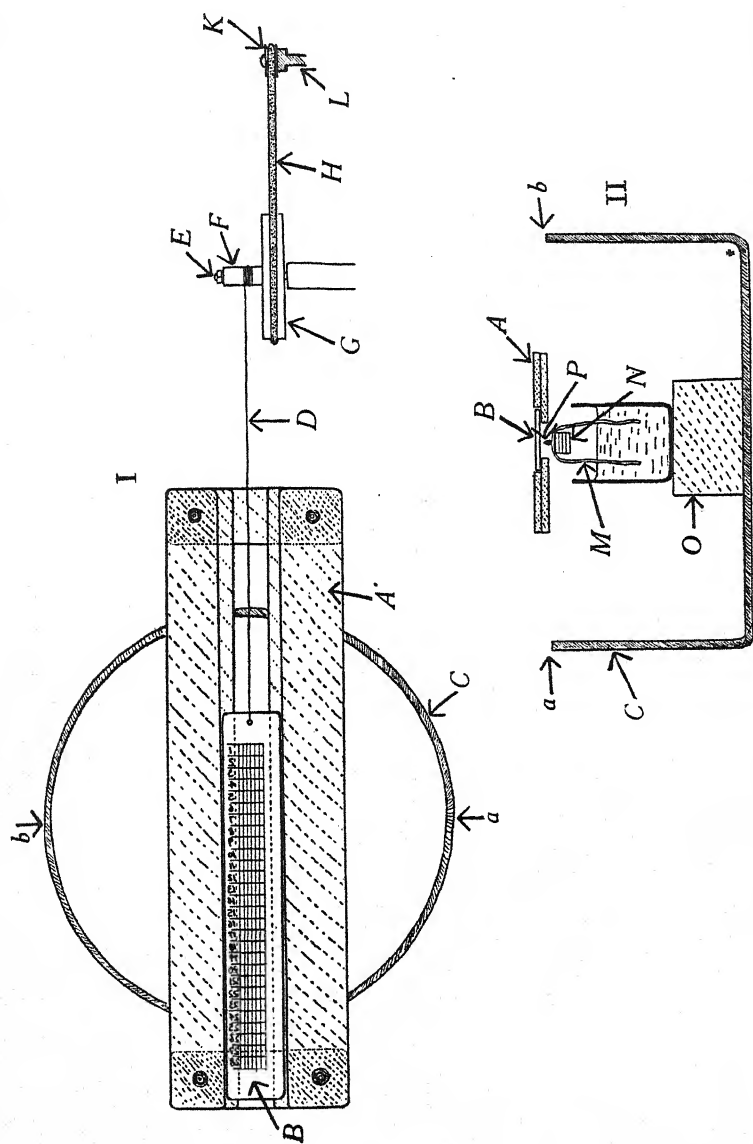
Fig. 6. Apparatus for obtaining continuous record of spore output. I, apparatus viewed from above; II, elevation along the line *a–b*. *A*, glass frame; *B*, recording slide; *C*, glass trough on which the frame rests; *D*, thread; *E*, axle on which rotate the winding axis *F* and its connected pulley wheel *G*; *H*, rubber band connecting the large pulley wheel to the small wheel *K* borne on the clock axis *L*; *M*, strip of filter-paper; *N*, pile of glass strips; *O*, block of wood; *P*, perithecium of fungus. × $\frac{3}{10}$.

work which forms a guide for the moving recording slide *B*. On one side of this slide a scale is etched. This is divided into twenty-eight sections by transverse lines, and seven longitudinal lines divide each section into six subsections. At one end of the slide a hole is bored and here a cotton thread is attached. The other end of the thread is fixed to the rotating axis *F*, a fine hole bored through this making attachment easy. The axis, *L*, of a double spring klinostat clock rotates once in 50 min. In order that the thread may be wound up sufficiently slowly this axis is fitted within a small pulley wheel, *K*, which by means of a rubber belt *H* drives the relatively large wheel *G*, the small winding axis *F* being concentric with this.

In the instrument used for this work the slide moved at the rate of 0·52 cm. (i.e. the length of one division on the slide) in 58 min. The fungus under consideration (*P*) is placed on moist filter-paper (*M*) resting on a pile of narrow glass slides (*N*), so that it comes within 2 mm. of the under surface of the recording slide. The distance of the fungus from this can be adjusted by varying the number of slides under the filter-paper. At the beginning of an experiment the re-cording slide is placed on the framework with the etched scale down-wards and adjusted so that the zero line of the scale is directly above the perithecium, or above the centre of the stroma in the case of a *Hypoxylon*. The thread connecting the slide with the winding axis should be tightly stretched. As the axis slowly rotates, the slide is dragged along above the fungus, and the spores discharged from this stick to the under-surface of the slide. At the end of 24 hours or so the recording slide is removed and replaced immediately by another. The spore record on the first slide can then be examined at leisure under the low power, the number of spores in each numbered division being counted. In the case of some forms in which the spore output is comparatively small all the spores on a slide can be counted. When, however, many thousands of spores are ejected per hour one has to rely on a method of estimation. The method adopted in this case is to take three high-power fields at random in each sub-section of each numbered section of the etched slide, and to count the spores thus seen. Since each numbered section is divided into six sub-sections (Fig. 6, I *B*), this means that the estimation for each section is based on the actual spores counted in eighteen high-power fields. Knowing the area of the microscope field and the area of each section, the total number of spores per section can be estimated.

Work on *Podospora curvula*, which has already been published, showed an obvious periodicity in its spore discharge. This work was

verified, using the continuous record method. Fig. 7 shows the spore chart for a single perithecium of the fungus, giving the exact time of discharge of each ascus (represented by a dot) during a period of 3 days. Similar results were obtained for the coprophilous fungus *Sporormia intermedia*. The investigation of the periodicity in discharge of this type was first suggested by the daily periodicity in discharge in other coprophilous fungi (e.g. *Ascobolus*, *Pilobolus*, *Coprinus lagopus*, etc.). It was considered likely that the non-coprophilous forms might show a continuous unvarying rate of spore
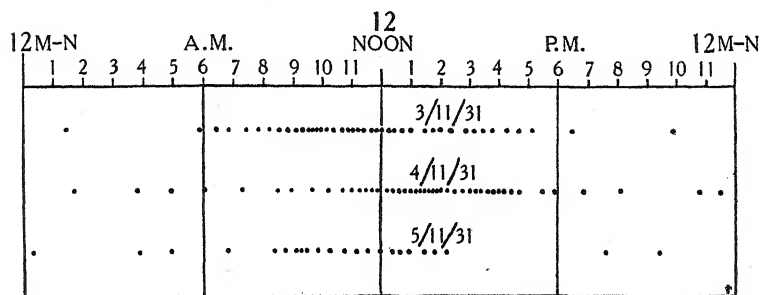
Fig. 7. Chart showing the spore output from a perithecium of *Podospora curvula* during 3 days. Each dot represents a single ascus discharge.

discharge such as Buller describes for Hymenomycetes. Accordingly records were made of spore discharge from certain lignicolous forms. Here too, however, spore output showed a diurnal periodicity.

In *Nectria cinnabarina* a state of affairs resembling that in *Podospora* was discovered. The results for two groups of perithecia are given in Tables II and III. The fungi were placed on damp filter-paper and subjected to the ordinary conditions of light and darkness.

TABLE II

Date November	Time	Spores	Temp. range ° C.
4–5	4.30 p.m.–8.30 a.m.	50	—
5	8.30 a.m.–5.30 p.m.	2060	—
5–6	5.30 p.m.–8.30 a.m.	0	—
6	8.30 a.m.–4.45 p.m.	1810	8–9
6–7	4.45 p.m.–8.30 a.m.	200	8–9
7	8.30 a.m.–7.0 p.m.	3100	8–9
7–8	7.0 p.m.–8.15 a.m.	10	7.5–9
8	8.15 a.m.–5.30 p.m.	3200	9–10

In Table III a dry stroma was used. It is to be noted that when a group of dry perithecia is first wetted, spore discharge may go on uninterrupted during both the day and night, but soon, under

ordinary conditions of illumination, the spore output settles down to its normal periodicity.

<div align="center">TABLE III</div>

Date December	Time	Spores	Temp. range ° C.
1–2	4.45 p.m.–9.0 a.m.	4520	7·5–9·5
2	9.0 a.m.–4.45 p.m.	2750	7·5–8·5
2–3	4.45 p.m.–8.30 a.m.	21	7·5–9
3	8.30 a.m.–4.45 p.m.	1570	7·5–8
3–4	4.45 p.m.–8.30 a.m.	0	6 –8
4	8.30 a.m.–4.30 p.m.	560	5·5–8·5
4–5	4.30 p.m.–8.30 a.m.	21	8·5–9
5	8.30 a.m.–4.30 p.m.	3620	7·5–9

Table IV gives the results of observations on the rate of discharge during the course of one day. Spore ejection reaches a maximum at the middle of the day.

<div align="center">TABLE IV</div>

Date Nov. 11	Time	Rate of discharge in spores per 10 min.
	9.30 a.m.	7
	10.30 a.m.	155
	11.30 a.m.	220
	12.30 p.m.	110
	1.30 p.m.	35
	2.30 p.m.	34
	5.30 p.m.	15
	8.0 p.m.	4
	9.0 p.m.–8.15 a.m.	0

Observations on *Hypoxylon coccineum* showed again a distinct diurnal periodicity, but in this case the maximum rate of discharge (which for a single small stroma involving 25–30 perithecia reached at times the rate of 1000 per min.), appears to occur late in the afternoon or evening. In this case only a few observations were made each day by exposing a slide about 1 mm. above the stroma and counting the spores deposited thereon in the course of one minute. The results are recorded in Table V and shown graphically in Fig. 8. It will be realised that the points in this figure are connected only to indicate roughly the probable shape of the curve. When placed in the dark room discharge still appeared to be periodic for the first few days, but after this it ceased.

In another species of *Hypoxylon*, *H. fuscum*, it was found, using the continuous record method, that spore discharge reached its maximum during the night. A curve for the spore discharge from a single stroma is given in Fig. 9. The fungus was placed in a laboratory facing north and during the period of the experiment
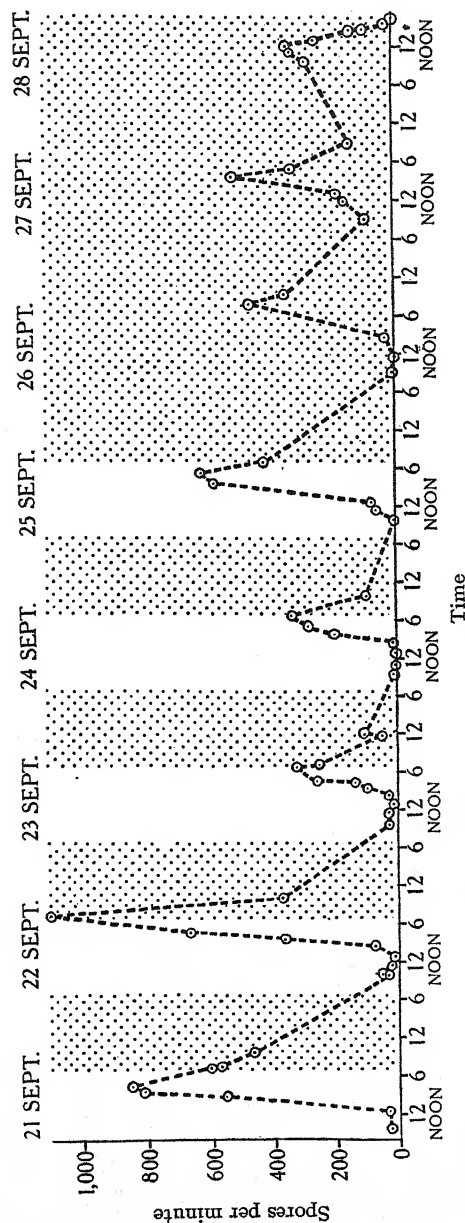
Fig. 8. Spore output curve for *Hypoxylon coccineum*. Points joined by dotted line to show probable form of the curve. Periods from sunset to sunrise and also the final period in the dark are stippled.

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the temperature varied less than 3° C. (17–19·5° C.). The diurnal periodicity in this case appears to be the direct result of an inhibiting action of light on spore ejection. To test this a stroma was used which had been showing the day-night periodicity for 5 days. It

TABLE V

Date September	Period	No. of spores discharged	Rate of discharge in spores per min.	Temp. ° C.
	In room facing north			
21	9.46– 9.47 a.m.	24	24	—
	9.54– 9.57 a.m.	64	21	—
	12.15–12.17 p.m.	72	36	—
	2.47– 2.48 p.m.	556	556	14·0
	3.27– 3.28 p.m.	812	812	13·8
	4.9 – 4.9½ p.m.	424	848	13·5
	7.7 – 7.8 p.m.	604	604	13·5
	7.24– 7.24½ p.m.	288	576	—
	9.45– 9.46 p.m.	468	468	12·5
22	9.40– 9.41 a.m.	56	56	11·0
	9.48– 9.50 a.m.	64	32	—
	9.57– 9.59 a.m.	104	52	—
	11.23–11.25 a.m.	40	20	12·4
	12.43–12.45 p.m.	16	8	12·2
	2.22– 2.24 p.m.	147	73	12·8
	3.32– 3.33 p.m.	360	360	12·9
	4.25– 4.26 p.m.	664	664	12·6
	7.15– 7.16 p.m.	1097	1097	12·8
	9.57– 9.58 p.m.	370	370	12·5
23	9.35– 9.36 a.m.	24	24	13·0
	11.34–11.35 a.m.	24	24	13·5
	12.50–12.51 p.m.	8	8	14·0
	2.18– 2.19 p.m.	24	24	14·5
	3.24– 3.25 p.m.	96	96	14·5
	4.20– 4.21 p.m.	132	132	14·3
	4.38– 4.39 p.m.	256	256	14·3
	6.44– 6.45 p.m.	320	320	14·0
	6.57– 6.58 p.m.	248	248	14·0
	11.40–11.41 p.m.	48	48	13·4
	11.47–11.49 p.m.	204	102	13·4
24	9.31– 9.33 a.m.	16	8	12·5
	10.45–10.56 a.m.	0	0	13·3
	11.2 –11.4 a.m.	0	0	13·3
	12.48–12.50 p.m.	0	0	13·8
	2.31– 2.33 p.m.	16	8	14·0
	3.46– 3.48 p.m.	392	196	13·6
	4.55– 4.56 p.m.	280	280	13·8
	6.27– 6.28 p.m.	336	336	13·3
	9.50– 9.51 p.m.	96	96	13·4
	9.59–10.0 p.m.	96	96	13·4
25	9.41– 9.43 a.m.	0	0	12·3
	11.17–11.19 a.m.	120	60	12·6
	12.24–12.25 p.m.	76	76	12·8
	3.22– 3.23 p.m.	583	583	13·0
	4.51– 4.52 p.m.	622	622	13·3
	6.40– 6.41 p.m.	423	423	13·8

Table V (*continued*)

Date September	Period	No. of spores discharged	Rate of discharge in spores per min.	Temp. ° C.
		In dark room		
26	9.27– 9.29 a.m.	24	12	13·0
	11.37–11.39 a.m.	0	0	13·0
	2.32– 2.34 p.m.	68	34	13·3
	7.32– 7.33 p.m.	463	463	13·6
	9.0 – 9.1 p.m.	352	352	13·0
27	9.45– 9.47 a.m.	184	92	12·8
	11.25–11.26 a.m.	160	160	12·9
	12.44–12.45 p.m.	184	184	12·9
	3.12– 3.13 p.m.	520	520	13·0
	4.30– 4.31 p.m.	336	336	13·0
	8.52– 8.53 p.m.	144	144	13·0
28	9.33– 9.34 a.m.	288	288	12·7
	10.59–11.0 a.m.	335	335	12·7
	11.55–11.56 a.m.	346	346	12·7
	12.51–12.52 p.m.	258	258	13·0
	2.24– 2.25 p.m.	144	144	13·0
	2.35– 2.37 p.m.	200	100	13·0
	3.35– 3.37 p.m.	64	32	13·0
	4.27– 4.30 p.m.	16	5	13·0
29	2.23– 2.24 a.m.	234	234	13·0

was placed in the dark room and illuminated by a 100-watt lamp placed above it, there being a distance of 14 cm. between the centre of the bulb and the fungus. The lamp was kept on for 24 hours and during this period no spores were discharged. The lamp was then turned off for 3 hours and discharge recommenced quite soon and became steadily more vigorous. After the 3 hours the lamp was again switched on. The spore output then began to fall and reached zero in a couple of hours. After 3 hours of illumination the lamp was once more switched off, and spore output began to increase. The result of this experiment is shown graphically in Fig. 10. In this experiment the temperature was not constant but fell, in the dark, to 17° C. and rose, with the lamp on, to a maximum of 27° C.

In another form investigated, *Melanomma* sp., a periodic discharge similar to that in *Hypoxylon fuscum* was observed, most of the spores being ejected during the night and very few in the course of the day.

The evidence presented above indicates clearly a general diurnal periodicity in the spore output of the Pyrenomycetes and light would seem to be the master factor in determining this periodicity. However, the effect of light is very different in different species, as in some light encourages while in others light inhibits spore ejection.
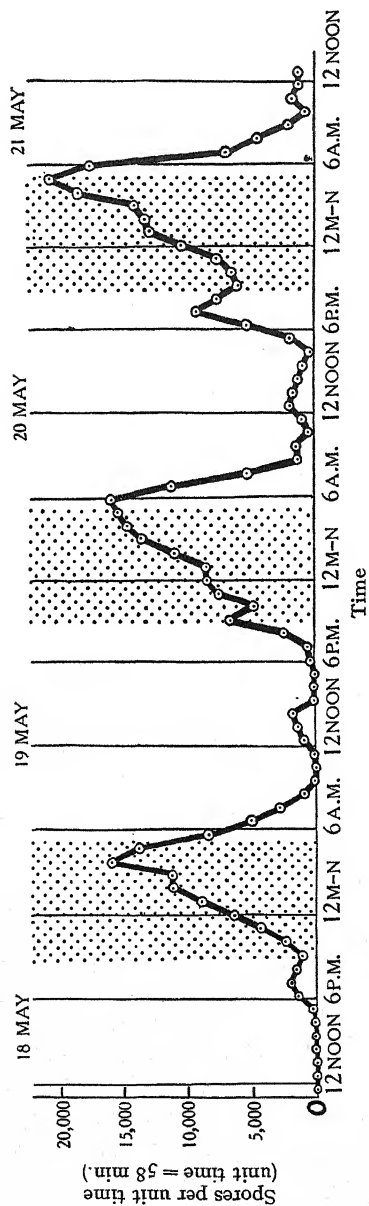
Fig. 9. Continuous record of spore output from a perithecial stroma of *Hypo-xylon fuscum*. Periods from sunset to sunrise are stippled.

IV. RATE OF SPORE DISCHARGE

From the results discussed in the foregoing section it will be evident that the rate of spore output undergoes a large amount of fluctuation. It is of interest, however, to examine the maximum
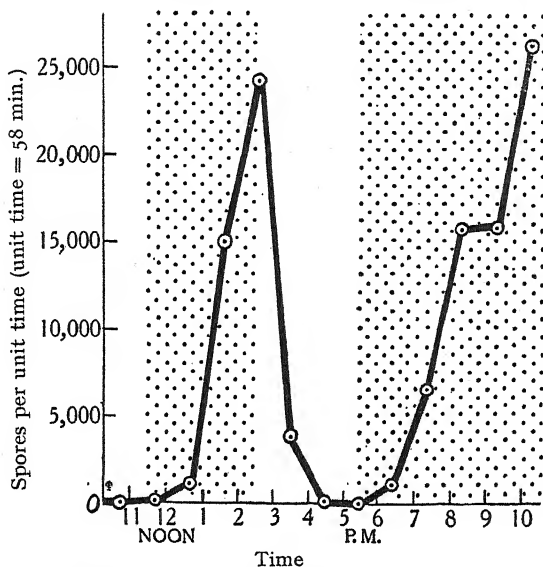
Fig. 10. Continuous record of spore output from a perithecial stroma of *Hypoxylon fuscum*. Stippled areas represent periods in the dark and unstippled areas periods of illumination by a 100-watt lamp 14 cm. above the fungus.

rates of discharge. Table VI gives the results of estimates, based on the observations of the present writer and on the recorded data of various observers, of the rates of spore output from a number of species reckoned in all cases as spores ejected per perithecium per hour.

TABLE VI

Species	Estimated spore output per hour per perithecium under favourable conditions	Observer
Podospora minuta	24	Griffiths [5]
P. curvula	40	C.T.I.
Sporormia intermedia	184	C.T.I.
Hypoxylon coccineum	1,800	C.T.I.
Diatrype disciformis	23,000	C.T.I.
Endothia parasitica	14,000	Rankin [10]
Nectria Peziza	456	Falck [4]
Hypomyces lactifluorium	352	Buller [2]

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THE RÔLE OF THE ORGANIC ACIDS IN PLANT METABOLISM

PART III[1]

By T. A. BENNET-CLARK

(With 4 figures in the text)

CONTENTS

I. The acid metabolism of the mould fungi

(a) Introduction

MANY mould fungi produce organic acids from the organic substrates on which they grow. Wehmer's classical work[44] established many of the conditions governing the processes of acid production by fungi. Since that time a voluminous literature has accumulated, and with the acquisition of new facts a number of hypotheses of the biological significance of the process have arisen. For the most part these hypotheses are closely similar to those introduced in explanation of the acid production of green plants; it is assumed by most workers that the chemical mechanism and biological significance of acid production is essentially the same in fungi as in the higher plants. It will be convenient to give a brief general statement of the typical behaviour of the mould fungi before dealing in detail with certain aspects of their acid metabolism.

[1] Parts I and II appeared in *New Phytologist*, **32**, 37, 128.

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Broadly speaking, one may state that Penicillia produce chiefly citric and gluconic acids, Aspergilli produce gluconic, citric, and oxalic acids, and Mucors produce succinic, fumaric and oxalic acids. But it is impossible to lay down rigid rules, as species differ considerably in the extents to which they accumulate these acids in their cultures. The nature of this variability will be dealt with in more detail later. It is very generally assumed that not only do different species differ considerably but that different strains of the same species exhibit marked differences in their metabolic behaviour.

The behaviour of typical acid-producing mould fungi grown on sugar solutions is as follows. Sugar is used up by the fungus, and when the necessary nutrient salts (K, Mg, N, P, S) are present the mycelium increases in size. At the same time organic acids are formed in the mycelium and these diffuse out into the medium in which the fungus is growing.

In consequence of this the acid concentration of the external medium rises. Eventually, after the greater part of the sugar in the medium has been used up, the acid which has accumulated outside the fungus begins to disappear, and is absorbed and converted by the fungus mycelium into other products. The facts should be borne in mind that the formation and disappearance of the acids are processes occurring inside the living mycelium. The enzymes associated with these changes have not been obtained either in the external solution or in preparations of the mycelium[1]. When supplied with a given initial quantity of sugar, the acid content of the culture solution plus fungus rises to a maximum value, and on continued starvation the acidity decreases roughly logarithmically. It has been shown by Currie, Molliard, and others that when more than one acid is produced, as in the case of *Aspergillus niger*, the maximum concentrations of the different acids occur at different times; thus gluconic acid first accumulates and as the concentration falls from the maximum value, citric acid accumulates and then disappears, and then oxalic acid accumulates and eventually it also disappears.

The simplest explanation of this phenomenon resembles that which has been discussed in connection with the acid accumulation of the Crassulaceae. The sequence of reactions:

$$\text{Sugar} \rightarrow \text{gluconic acid} \rightarrow \text{citric acid} \rightarrow \text{oxalic acid} \rightarrow CO_2$$
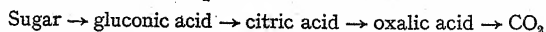
would result in accumulation of gluconic acid to a maximum value followed by a decrease in concentration and similar rises in citric

[1] Glucose-oxidase which converts glucose into gluconic acid has been isolated from *A. niger* (Muller(36)).

and oxalic acid concentrations to maximum values followed by gradual falls to zero, if the values of the velocity constants have the relative magnitudes $k_1 > k_2 > k_3 > k_4$.

In a very large number of the researches on the acid production of mould fungi, calcium carbonate has been added to the culture solution. The effect which this has on the processes taking place is that any acid which diffuses out of the fungus into the solution reacts with the calcium carbonate to form the calcium salt of the acid and CO_2 is liberated. Under these conditions considerably larger yields of the acid (in the form of calcium salt) are obtained, so it appears that the acid is protected from further conversion into other products by its conversion into a salt.

The metabolic processes are profoundly affected by external conditions of which the more important are enumerated below.

(i) *Carbon dioxide and oxygen concentration.* In most work on the metabolism of moulds no attempt has been made to control the concentrations of these gases surrounding the culture. When the cultures are grown in flasks plugged with cotton-wool the oxygen concentration inside becomes considerably lower and the CO_2 concentration correspondingly higher than in ordinary air. Obviously the concentrations of these gases depend on the rate of respiration of the fungus and on the degree of packing of the cotton-wool plug. The present writer has found oxygen concentrations as low as zero, and CO_2 concentrations of 25 per cent. in culture flasks of *Aspergillus niger* plugged tightly with cotton-wool. Under such conditions, the concentrations of these gases do not remain constant during a long-continued experiment, as they are largely determined by the rate of respiration of the fungus.

The same remarks also apply to the method employed by Raistrick and his colleagues. They grew mould fungi in flasks through which air could be aspirated, but, instead of leading air or any other desired gas mixture through the flasks continuously, they passed about 500 c.c. of air (free from CO_2) through the flasks for 1 hour each day and then shut off the air stream for the remaining 23 hours, at the end of which time all the oxygen had been used up and replaced with CO_2. Thus aerobic conditions obtained for a short period each day and were gradually (and at unknown times) replaced by conditions of anaerobiosis and CO_2 narcosis.

The products formed under such conditions differ from those formed when the fungus is grown under aerobic conditions: this is made clear by data given in Table I. The quantities of carbon con-

verted by the fungus to various forms are given as percentages of the total glucose carbon which has been used by the fungus: glucose was the sole carbon compound initially present.

TABLE I

Products formed from glucose by Aspergillus niger *under aerobic conditions, and under conditions of narcosis alternating with aerobic conditions of unknown length*

	Narcosis (Raistrick(39))	Aerobic conditions (Bennet-Clark, unpublished)
Carbon in mycelium	13·1	56·8
Carbon as CO_2	31·4	28·4
Carbon as non-volatile acids (oxalic, citric, etc.)	15·6	11·6
Carbon as non-volatile neutral compounds	14·0	2·8
Carbon as volatile compounds (chiefly alcohol)	25·5	0·9
Total carbon used (supplied in the form of glucose)	100	100

The most striking difference is that under conditions in which accumulations of CO_2 occur alcohol and other neutral compounds such as glycerol and mannitol accumulate in the solution. Under aerobic conditions these compounds account for a very small part of the sugar used by the fungus: on the other hand the weight of the mycelium is much greater partly owing to the high glycogen content. The absence of alcohol production under strict aerobic conditions may be due to the conversion of alcohol or some precursor of alcohol into glycogen.

Except when strictly aerobic conditions have been obtained by leading a rapid stream of sterile air through the culture flask, the compositions of the gas mixtures have not been controlled or kept constant during experimental work, as it has been assumed quite incorrectly that the obstruction to gaseous diffusion offered by the cotton-wool bungs in the necks of culture flasks is negligible (cf. Raistrick(39), who terms this unrestricted aeration). The effects of anaerobic conditions or narcosis on the acid metabolism have not been adequately investigated yet, but preliminary results indicate that the fate of the commoner acids is considerably affected by the carbon dioxide content of the medium.

(ii) *The effect of nutrient salts.* Molliard(33) first discovered that *Sterigmatocystis nigra* (=*Aspergillus niger*) produced gluconic acid

when grown on culture solutions containing abnormally small quantities of nitrogenous salts. Some of his results, which are discussed on p. 204, are illustrated graphically in Fig. 1. If will be noted that reduction of the nitrogen supply causes increased accumulation of gluconic acid and decreased accumulation of citric acid.
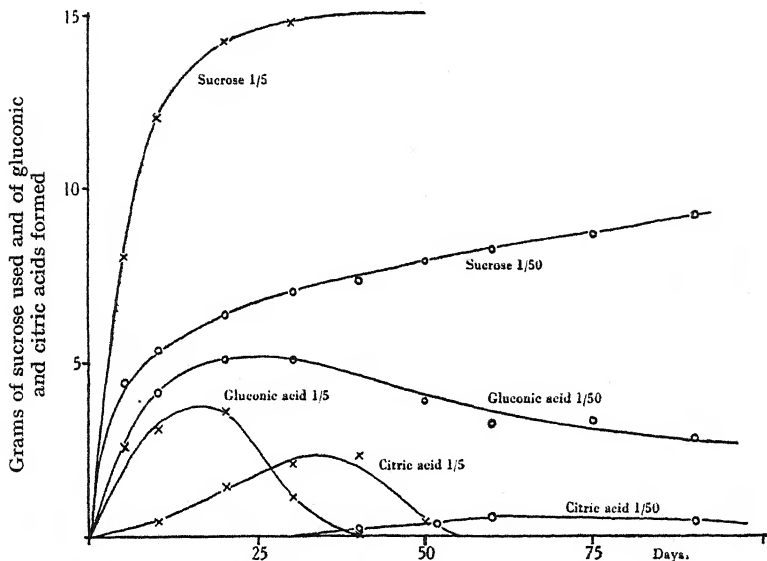
Fig. 1. The quantities of sucrose, gluconic, and citric acids in 100 c.c. of culture solutions (original sucrose content 15 grm.) containing respectively 1/5th and 1/50th of the "normal" amount of nitrogenous salts. *Aspergillus niger* (Molliard (34)).

Somewhat similar results were obtained by Bernhauer. He found that the yields of citrate are greater the higher the nitrogen content and the higher the temperature. These conditions cause the yield of gluconate to be negligibly small. The significance of these effects of nitrogen supply and temperature is as yet quite uncertain.

(b) *The Franzen-Schmitt hypothesis of citric acid formation*

This hypothesis was outlined in Part I of this paper. Challenger, Walker and Subramaniam(18, 43) accepted this view and held that the .process of sugar breakdown occurring in *Aspergillus* was as follows:

Sugar → gluconic acid → saccharic acid → citric acid → acetonedicarboxylic acid → malonic + acetic acids → glycollic acid → glyoxylic acid → oxalic acid → carbon dioxide.

The nature of the evidence both in favour of this view and against it is unsatisfactory, and does not permit a definite decision to be made. As early as 1924, before this hypothesis had been propounded in its entirety as a result of the work of Franzen and Schmitt (26), Butkewitch (14) decided that citric acid was not produced from gluconic acid by *Aspergillus niger*. He held (13, 14) that the intermediate stages in the conversion of glucose to citric acid involved the formation of glucuronic acid, which formed a 5-C ring compound by an internal condensation, and that this compound was converted into citric acid (see Part I, p. 58). He showed that the *Aspergillus* strain which he used produced both citric and gluconic acids when grown on glucose solutions, but that when it was grown on solutions of calcium gluconate no citric acid was formed. It appeared to follow that citric and gluconic acid production were two independent processes.

Actually this conclusion is not entirely justified, as it was shown that the presence of calcium carbonate in the cultures of this fungus on glucose greatly influenced the nature of the acids formed. The nature of the effects are indicated in the table given below:

	With CaCO$_3$	Without CaCO$_3$
Yield of gluconic acid	0·840	0·280
Yield of citric acid	0·051	0·727
Yield of oxalic acid	0·474	0·000

The obvious explanation of those results is that calcium carbonate "traps" the gluconic acid and oxalic acid formed. In other words, the free acids are convertible by the fungus into other products but the calcium salts are not so convertible. The high yield of citric acid when the gluconic acid is not trapped might obviously be due to the conversion of gluconic acid to citric acid. The fact that citric acid is not formed when the fungus is grown on calcium gluconate is not in opposition to this view, and is somewhat confirmatory of it, since it suggests that the calcium carbonate does in fact act as a "trap" for gluconic acid in the manner indicated. This matter would have been settled if the fungus had been grown on gluconic acid instead of its calcium salt, but this was not done and apparently has not been done since.

Other investigators, working presumably with different strains of *Aspergillus niger*, have, however, found that the Aspergilli used by them produced citric acid when grown on solutions of calcium gluconate. Thus Challenger and his co-workers (18) isolated 1·2 grm. of citric acid and 3·7 grm. of calcium saccharate from a culture of

Aspergillus niger grown for 5 days on a solution containing 20 grm. of calcium gluconate.

They also showed that the same fungus produced small amounts of citric acid when grown on solutions of potassium hydrogen saccharate. These facts were regarded as evidence of the occurrence of the reactions:

Gluconic acid → saccharic acid → diketoadipic acid → citric acid.
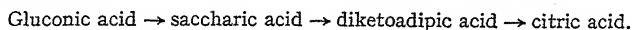
In order to establish the view that the conversion of glucose to citric acid followed this course (the first stage being its oxidation to gluconic acid), it would be necessary to show that as large a yield of citric acid is obtained when the fungus is grown on gluconic acid or saccharic acids as when grown on an equivalent solution of glucose.

Bernhauer's results (5) suggest that this does not occur; in fact no citric acid was obtained when the fungus was grown on solutions of potassium hydrogen saccharate although many citric acid producing strains of *Aspergillus niger* were used. These results indicate that saccharic acid is certainly not an intermediate product in the conversion of glucose to citric acid; but evidence that gluconic acid is not an intermediate product in this change has not been obtained.

Experiments carried out to test the possibility that gluconic acid is formed as an intermediate in the conversion of glucose into citric acid convinced Bernhauer (4) that this might actually occur.

It was shown that the rate of citric acid production from calcium gluconate solutions was as great, other conditions being equal, as from glucose solutions containing calcium carbonate. In this work, however, ready-grown mats of fungus mycelium were supplied with the solutions, and it is not therefore proven that the citric acid produced did not originate from polysaccharides stored in the mycelium.

On the other hand, Bernhauer has clearly shown that the strains of *Aspergillus* which conduct these changes are unable to convert saccharic acid or saccharates into citric acid. The strain of *Aspergillus* which converted potassium hydrogen saccharate into citric acid, according to Challenger and his co-workers, was found by Bernhauer to have lost this power four years later when he reinvestigated the problem. This fact makes acceptance of the Franzen-Schmitt hypothesis difficult.

Bernhauer's and Molliard's results, however, are difficult to reconcile with any other view than that conversion of sugar into gluconic acid occurs and that this is further converted into, eventually, citric acid.

(c) *The effects of nitrogen compounds on gluconic acid formation*

Molliard, who first discovered the gluconic acid production of mould fungi(33), also showed that the quantities of gluconic and citric acids accumulated in the media on which the fungus grows are much influenced by the quantities of the nutrient salts supplied.

Some results taken from Molliard(34) have been illustrated in the graphs of Fig. 1. Molliard grew his cultures of *Aspergillus* (*Sterigmato-cystis nigra*, presumably *A. niger*) in a standard nutrient of the composition: sucrose 15 grm., KH_2PO_4 0·142 grm., $MgSO_4$ 0·061 grm., $ZnSO_4$ and $FeSO_4$ 0·007 grm., NH_4NO_3 0·534 grm., made up to 150 c.c. with water. Solutions otherwise similar but containing 1/5, 1/25, 1/50, etc., of the quantity of ammonium nitrate in the standard solution were also used. No calcium carbonate was added to these cultures.

Fig. 1 records the quantities of citric and gluconic acid present and the quantity of sucrose which has been used up, at the times given by the abscissae. Two series of curves are given, one referring to the culture on the standard solution with 1/5 of the "normal" nitrogen content and the other to a solution with 1/50 of the normal nitrogen supply. With the latter solution, it will be noted that the sugar is rather slowly used up and that large quantities of gluconic acid accumulate. After about the 25th day this gluconic acid decreases in concentration. An inconsiderable accumulation of citric acid, which reaches its maximum concentration about the 75th day, occurs. With the larger nitrogen supply, the sugar disappears very rapidly, the gluconic acid concentration rises to a relatively low maximum value attained at about the 15th day. By the 35th day all the gluconic acid has disappeared and the citric acid concentration has risen to its maximum value, and by the 55th day all the citric acid has disappeared.

Beyond pointing out that the reduced nitrogen supply favours the gluconic and disfavours the citric acid formation, Molliard does not discuss the significance of the results. We may, however, note that these results are quite in agreement with the hypothesis that gluconic acid is an intermediate product in the oxidation of sugars to citric acid, as indicated on p. 198. To bring these results of Molliard into line with that hypothesis it is necessary to postulate in addition that the velocity constant of the conversion of gluconic into citric acid is increased in the presence of larger nitrogen supplies. This would evidently result in larger yields of citric and smaller of gluconic acid.

A point of some interest is raised by the results of the experiment where the nitrogen supply was 1/50 normal. It will be noted from the graph that during the first 15 days the weight of gluconic acid in the culture was about 80 per cent. of the weight of sucrose which had disappeared. Now the oxidation of 1 mol of glucose (180 grm.) can yield a maximum of 1 mol of gluconic acid (196 grm.), so it follows that the fructose part of the sucrose molecule is also converted into
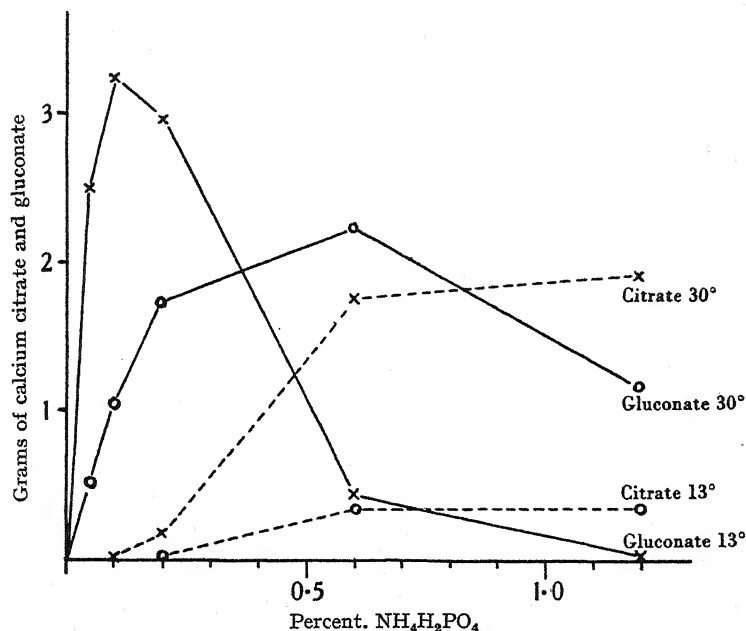
Fig. 2. Yields of calcium citrate and gluconate obtained from cultures of *Aspergillus niger* grown for 5 days on 50 c.c. solution containing 5 grm. sucrose and the quantities of nitrogen salts indicated as abscissae. (Bernhauer (3)).

gluconic acid. The yield of gluconic acid is roughly 150 mols for every 100 mols of sucrose used.

The general conclusion that high nitrogen favours citric and disfavours gluconic acid accumulation and *vice versa* has been confirmed by Bernhauer (3). His experiments were conducted in a totally different manner. All his cultures were grown in solutions of standard composition containing different quantities of nitrogenous salts and also calcium carbonate. The experiments were stopped and the cultures analysed on the 5th day after the start.

The yields of calcium citrate and calcium gluconate are given in Fig. 2 plotted against the percentage of the nitrogen-containing salt ($NH_4H_2PO_4$ in this case). One series of cultures was grown at 13° C., and the other at 30° C. The yields of calcium citrate are in all cases negligible at low temperatures, and the yields of calcium gluconate are relatively large. At the higher temperature the yield of citrate is good and that of gluconate negligible when the nitrogen supply is high (over 0·6 per cent.) and conversely with low nitrogen supply (0·05–0·2 per cent. of ammonium phosphate) the yield of citric acid is negligible and that of gluconic acid relatively large. These effects appear to be independent of the nature of the nitrogen compound supplied, various nitrates and ammonium salts all brought about similar changes.

(d) The conversion of 2-C compounds into 4-C and 6-C compounds

Butkewitch and Fedoroff (15–17) showed that various species of *Mucor* produced succinic and fumaric acids when supplied only with acetates or alcohol as source of carbon. *Mucor stolonifer* when supplied with sugar accumulates calcium fumarate in cultures containing calcium carbonate. The later work of Butkewitch and Fedoroff showed that calcium succinate also accumulates and that about 13 per cent. of the total acid formed is succinic and the rest fumaric. They showed that succinic acid was formed predominantly when acetic acid was supplied as the source of carbon instead of sugar, and that quite small amounts of fumaric acid accumulated. Roughly 15 per cent. of the acetic acid consumed by the fungus was apparently converted into fumaric and succinic acids after 30 days' growth of the culture. About 81 per cent. of the total fumaric + succinic acid formed was found to be succinic acid.

Curiously enough, when alcohol is supplied as the sole source of carbon, fumaric together with only small traces of succinic acid are formed (as calcium salts). It was also shown that when cultures were supplied with succinic acid as the sole source of carbon considerable quantities of oxalic acid (as Ca salt) were formed, but that only small traces of fumarate accumulated. Furthermore, in the presence of sugar, little oxalic and much fumaric acid are formed. The conversion of succinic to fumaric acid is thus seen to be dependent on the presence of certain products of glycolysis which may act as hydrogen acceptors.

It is suggested that the formation of fumaric and succinic

acids from sugar under aerobic conditions involves the following reactions:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH,$$
$$2C_2H_5OH + 2O \rightarrow 2CH_3COOH + 2H_2O,$$
$$2CH_3COOH + O \rightarrow COOH.CH_2.CH_2.COOH + H_2O,$$
$$COOH.CH_2.CH_2.COOH - 2H \rightarrow COOH.CH = CH.COOH.$$
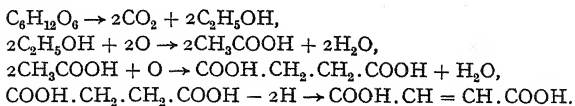
It is well known that succinic acid is also formed in small quantities under anaerobic conditions during the alcoholic fermentation which both yeast and *Mucor* bring about, and Butkewitch and Fedoroff suggest that it arises in a manner similar to that given above.

Butkewitch and Fedoroff suggest that part of the fermenting sugar reacts according to the Neuberg schema thus:

$$2C_6H_{12}O_6 + H_2O = 2CO_2 + 2C_3H_5(OH)_3 + C_2H_5OH + CH_3COOH,$$
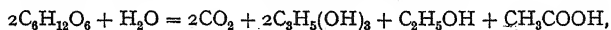
and that the acetic acid is converted into succinic acid by the Thunberg reaction:

$$2CH_3COOH - 2H = COOH.CH_2.CH_2.COOH.$$

The two atoms of hydrogen are supposed to be accepted by a molecule of methyl-glyoxal with the resultant formation of another molecule of glycerol. In order to produce one molecule of succinic acid, two molecules of acetic acid will be required, and this will necessitate the using up of four molecules of glucose according to the Neuberg reaction given above. The dehydrogenation of the acetic acid to succinic acid requires another half glucose molecule to produce the methylglyoxal required to accept the hydrogen. The whole process can therefore be summarised thus:

$$9C_6H_{12}O_6 + 2H_2O = 8CO_2 + 10C_3H_5(OH)_3 + 4C_2H_5OH + 2C_2H_4(COOH)_2,$$
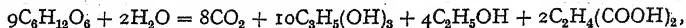
the ratio of number of molecules of succinic acid to number of molecules of glycerol formed is $1:5$. The observed ratios of the molar concentrations of these substances in fermenting yeast and *Mucor* cultures remain remarkably constant during the development of the culture though the absolute concentrations change considerably; the ratio demanded by Butkewitch and Fedoroff's hypothesis is approximately attained. These authors point out that their hypothesis is in part a return to the older view of Pasteur that the succinic acid formed in alcoholic fermentation is derived from sugar.

It is at present much more generally believed that the succinic acid formed in alcoholic fermentation is formed from glutamic acid. That this change can take place appears probable from the work of Ehrlich who showed that the yield of succinic acid is increased when

glutamic acid is added to fermenting yeast cultures. Some of
Ehrlich's results[25] are given in Table II. It is, however, remarkable
that in the absence of sugar almost no succinic acid is formed even
when glutamic acid is present.

TABLE II

*Yields of succinic acid from fermenting yeast (100 grm.)
in water (2000 c.c.) with various substances added*

Amount of sugar grm.	Amount of nitrogenous substances	Yield of succinic acid grm.
200	None	1·20
200	1 grm. glutamic acid	1·67
200	2 grm. glutamic acid	2·33
200	5 grm. glutamic acid + 5 grm. ammonium phosphate	0·21
0	5 grm. glutamic acid	0·05
0	None	0·01

Butkewitch's hypothesis has been strikingly confirmed by the
recent work of Lundsgaard[31]. The starting-points of Lundsgaard's
work were the observations that yeast suspensions supplied with
alcohol and oxygen have a respiratory quotient of about 0·33. The
complete combustion of alcohol to CO_2 and water (reaction (2) below),
gives a ratio CO_2/O_2 of 0·66. Part of the alcohol is therefore clearly
oxidised to other substances, of which carbohydrate (glycogen) ap-
peared to be the most likely to be formed. The combination of the
two reactions given below, in which a quarter of the alcohol is
completely oxidised and the remainder resynthesised to carbo-
hydrate, evidently would account for the observed R.Q. of 0·33:

$$(1) \quad 3C_2H_5OH + 3O_2 = C_6H_{12}O_6 + 3H_2O.$$
$$(2) \quad C_2H_5OH + 3O_2 = 2CO_2 + 3H_2O.$$
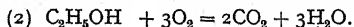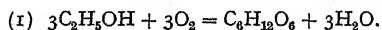
It is also known that resynthesis of glycogen in muscle is pre-
vented by poisoning with iodacetic acid. Lundsgaard showed that
the R.Q. of yeast suspension in alcohol solutions was roughly 0·33
both in unpoisoned yeast and in suspensions poisoned by iodacetic
acid. If resynthesis of glycogen in yeast is stopped by iodacetic acid
as it is in muscle the explanation of the low R.Q. might have to be
sought in the oxidation of alcohol to other products than carbo-
hydrate. Actually, experiment showed both that organic acids are
produced in addition to carbohydrate by yeast suspensions supplied
only with alcohol, and also that carbohydrate resynthesis does not
occur in poisoned yeast.

TABLE III

Metabolic products formed by yeast suspension (30 mg. yeast in 1·5 c.c. phosphate buffer + alcohol)

	Poisoned with $M/1000$ iodacetic acid	Normal
Respiratory quotient	0·34	0·33
Oxygen used in 3 hours, cu.mm.	405	702
Carbon dioxide evolved in 3 hours, cu.mm.	131	244
Ether soluble acid formed, mg. equiv.	4·6	4·6
Alcohol used, mg. per 100 c.c. of the suspension	31·0	56·0
Alcohol oxidised completely (per 100 c.c.) calculated from CO_2 output	9·5	17·1
Alcohol converted into ether-soluble acid (per 100 c.c.) calculated from amount of acid formed on assumption that 1 mol. alcohol produces 1 equiv. of acid	21·1	21·1
Alcohol oxidised to carbohydrate (per 100 c.c.) obtained by difference	0·4	16·8
Molecules of O_2 required to convert 1 mol. of alcohol into 1 equiv. acid	1·36	1·48

A large part of the alcohol oxidised is converted into organic acids. The amount of oxygen used in the conversion of alcohol to carbon dioxide and carbohydrate can be calculated and the difference between this quantity and the total oxygen absorbed gives the amount absorbed in the formation of organic acids. The amount of oxygen required in order to oxidise alcohol to acetic acid is 1 molecule per molecule of alcohol, to form succinic acid 1·25 molecules, to form malic or fumaric acids 1·5 molecules, and to form glycollic acid 2·0 molecules per molecule of alcohol oxidised. The observed oxygen intakes in this case are 1·36 and 1·48 molecules per molecule of alcohol, and it therefore seems probable that the acid formed is a mixture of the 4-C acids just mentioned. It is well known that both succinic and malic acids are formed in normal fermentation (29). This work in conjunction with that of Butkewitch and Fedoroff suggests that these acids are formed from carbohydrate sources rather than from proteins, as Kostytchev and Frey (29) suggested chiefly on the basis of Ehrlich's work. The fact that alcohol is converted into these acids but not into carbohydrate in yeast poisoned with iodacetic acid is a result of some importance as it suggests that the reaction which occurs is the direct change alcohol → 4-C acids and not alcohol → carbohydrate → 4-C acids.

Just as the Mucors and yeasts have been shown to convert 2-C

compounds into the 4-C acids, so it has been recently shown that *Penicillium* (Chrząszcz and Tiukow [22]), and *Aspergillus* (Bernhauer and Siebenäuger [6]) convert 2-C compounds into citric acid. Chrząszcz and Tiukow, who first noted the conversion of acetates into citrates by a species of *Penicillium*, regarded the change as analogous to the Thunberg reaction. The reactions are as follows:

$$
\begin{array}{ccc}
CH_3COOH & CH_2COOH & CH(OH).COOH \\
\quad\quad -2H \rightarrow \quad | \quad +O \rightarrow & | \\
+CH_3COOH & CH_2COOH & CH_2.COOH \\
\text{Acetic acid} & \text{Succinic acid} & \text{Malic acid}
\end{array}
$$

$$
\begin{array}{cccc}
CHOH.COOH & CH_2.COOH & & CH_2.COOH \\
| \quad\quad -2H \rightarrow & C(OH).COOH & \text{or} & CH_2.COOH \quad -2H \rightarrow \\
CH_2.COOH & | & & | \\
+CH_3.COOH & CH_2.COOH & & +CH_3.COOH \\
\text{Malic + acetic acids} & \text{Citric acid} & & \text{Succinic + acetic acids}
\end{array}
$$

$$
\begin{array}{cc}
CH_2.COOH & CH_2.COOH \\
\rightarrow CH.COOH \quad\quad \rightarrow & C(OH).COOH \\
| & | \\
CH_2.COOH & CH_2.COOH \\
\text{Tricarballylic acid} & \text{Citric acid}
\end{array}
$$
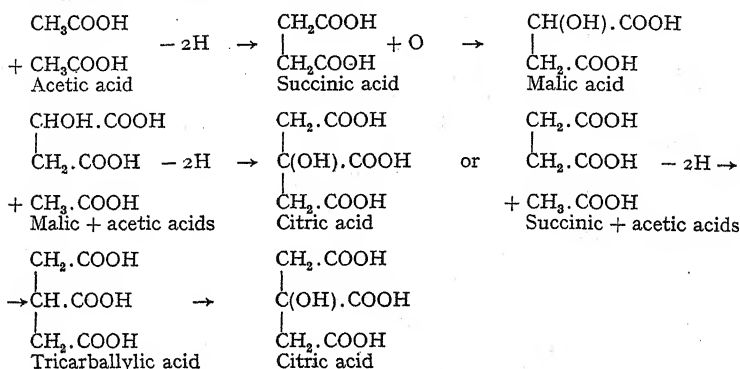
It was not at first possible to decide whether greater probability attached to the view that malic and acetic acids reacted to give citric acid, or whether tricarballylic acid is first formed and then oxidised. More recent work (Bernhauer and Böckl [8]) shows that an equilibrium between aconitic and citric acids occurs in *Aspergillus* comparable to the fumaric-malic acid equilibrium which is catalysed by fumarase. This suggests, though it does not establish, that the reactions below are also possible

$$
\begin{array}{ccc}
CH_2.COOH & CH.COOH & CH_2.COOH \\
CH.COOH \quad -2H \rightarrow & C.COOH \quad +H_2O \rightleftharpoons & C(OH).COOH \\
CH_2.COOH & CH_2.COOH & CH_2.COOH \\
\text{Tricarballylic acid} & \text{Aconitic acid} & \text{Citric acid}
\end{array}
$$
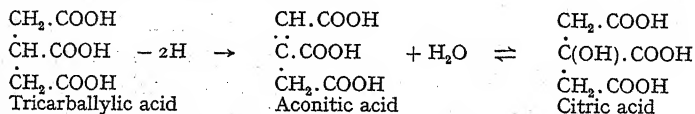
or yet again the further alternative is possible that fumaric and acetic acids give rise by the Thunberg reaction to aconitic acid directly.

The yields of citric acid obtained from acetates and alcohol are of the same order of magnitude as the yields of the 4-C acids obtained in the Mucors and yeasts. According to the findings of Chrząszcz and Tiukow [22, 23] different species of *Penicillium* differ greatly in their power of citric acid production from 2-C compounds. Similar results were obtained by Bernhauer and Siebenäuger working with *Aspergillus niger*: out of a total of twenty-eight different strains

examined, eight produced citric from acetic acid. The largest yields obtained were about 16 per cent. of the total acetic acid used, in general about 30–40 per cent. of the acetic was also converted into oxalic acid. It is noteworthy that the most active citric acid producing strain formed only traces of citric acid when supplied with malic or succinic acid, and that the yield was only about 3 per cent. when fumaric acid was supplied.

Lundsgaard's work, however, makes it appear improbable that carbohydrate is an intermediate product in the conversion of alcohol and acetic acid into citric acid. These workers agree in considering that the process by which citric acid is formed from sugars is as follows:

Glucose → methyl-glyoxal → pyruvic acid → acetaldehyde → acetic acid → citric acid.
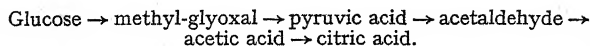
(e) *The conversion of 2-C compounds into oxalic acid*

Aspergillus niger and many other mould fungi produce oxalic acid in considerable quantities when supplied with a variety of organic substrates. Considerable amounts are formed when carbohydrates or proteins are supplied, but it is striking that few of the more simple carbon compounds which can be utilised by the fungus are converted into oxalic acid. Raistrick and Clark (38) found that *Aspergillus niger* grew (in the presence of the essential nutrient salts) on the following acids: succinic, fumaric, maleic, malic, tartaric, lactic, pyruvic, malonic, acetic, glycollic, glyoxylic, and formic. Oxalic acid is produced in quantity (yields of about 30–40 per cent. of the maximum possible) in cultures on solutions of succinic, fumaric, malic, or acetic acids, but it is not formed or is formed only in very small traces when any of the other acids just mentioned are supplied.

It is particularly striking that the 3-C acids, and glycollic and glyoxylic acids are not converted into oxalic acid. It is not pointed out by Raistrick and Clark that this fact indicates that the direct oxidation of acetic to oxalic acid does not occur. This change could only occur thus:

$$CH_3COOH \rightarrow CH_2OH.COOH \rightarrow CH=O.COOH \rightarrow COOH.COOH$$
Acetic acid   Glycollic acid   Glyoxylic acid   Oxalic acid
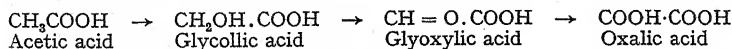
In fact their observation rules out the possibility that the last stage in the formation of oxalic acid is oxidation, since glyoxylic acid would naturally be an intermediate product in oxalic acid formation if this were the case; glyoxylic acid would therefore be converted into oxalic acid by the fungus which, experiment shows, does not occur.

These results have been confirmed by Bernhauer and Scheuer[7] who showed that three strains of *Aspergillus niger*, which gave yields of about 40 per cent. of oxalic acid when supplied with acetic acid, produced no oxalic acid when supplied with glycollic acid. Citric acid was produced from glycollic acid, and both citric and oxalic acids were produced from succinic acid. These results, in conjunction with Raistrick and Clark's, seem to show that the conversion of acetic to oxalic acid takes place according to the following scheme:

$$2CH_3COOH - 2H \rightarrow (CH_2.COOH)_2 \rightarrow COOH.COOH.$$
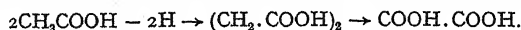
The conversion of succinic to oxalic acid might be expected to take place thus:

Succinic acid $- 2H \rightarrow$ fumaric acid $\rightarrow$ oxalacetic acid $\rightarrow$ oxalic $+$ acetic acid.
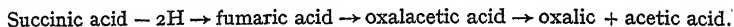
The last change, the "acid hydrolysis" of oxalacetic acid, has been discussed in the first part of this paper. This view of the mode of origin of oxalic acid has the advantage that it readily explains how acetic, succinic, fumaric, and malic acids are the only acids of the series examined by Raistrick and Clark which give rise to oxalic acid.

These results justify the conclusion that the 2-C compounds are not, in general, oxidised (by *Aspergillus niger*) to oxalic acid, and therefore are not oxidised to carbon dioxide. They are anabolised to 4-C compounds. The fate of glycollic and glyoxylic acids will be dealt with in greater detail in a later section. No direct evidence that oxalic acid is ever formed in organisms by oxidation of other 2-C compounds is available, since it is at present impossible to show that an apparent conversion of a 2-C compound to oxalic acid does not involve previous conversion into more complex compounds.

(f) The significance of the maximum yields of acids formed in fungus cultures

When calcium carbonate is present in cultures grown on sugar the yield of the acids produced by the fungus is much higher than in the absence of calcium carbonate, since the free acids react to give calcium salts which may escape further decomposition, whilst the free acids are liable to be converted into other products. Obviously, if the Neuberg scheme of reactions holds good for the processes of sugar breakdown, and the resulting 2-C compounds are converted into 4-C acids and citric or oxalic acid, it follows that, at the most, two-thirds of the sugar is converted into the organic acids and one-third into carbon dioxide. So, if the yield of acid found experi-

mentally exceeds the yield demanded by the Neuberg schema, this view of the mode of formation of the acids will require modification.

Table IV

Maximum yields of certain acids formed by mould fungi in presence of calcium carbonate. Results are given as weight of acid formed per 100 grm. of sugar consumed

Acid	Yield observed	Maximum yield possible on basis of Neuberg reactions	Author
Citric acid	73·6	75·0	Bernhauer, Böckl, and Siebenäuger*[9]
	67·8	—	—
	74·3	—	—
	68·8	—	—
	68·2	—	—
	85·5	—	Butkewitch[13]
Fumaric acid	63	64·5	Wehmer[47]
	59	—	—
Oxalic acid	81	100·0	—
Gluconic acid	81	72·6†	Molliard[34]
	69	—	Bernhauer[4]
	54	—	May, Herrick, Thom, and Church[32]

* In estimating the "sugar consumed" these authors subtracted the weight of mycelium from the actual sugar consumption in order to obtain "sugar converted to acid."

† 109·0 if 1 mol sugar → 1 mol acid.

It will be noted that in the cases recorded here the yields are surprisingly close to the maximum yield compatible with the occurrence of the Neuberg reactions as the first stage in sugar breakdown. In two cases the yields are considerably larger. Butkewitch's yield of 85·5 per cent. of citric acid produced by *Aspergillus niger* has been cited as evidence against the Chrząszcz-Tiukow hypothesis of citric acid production. That is, however, the sole record of such a high yield of citric acid. Molliard's high yields of gluconic acid may perhaps indicate that this acid is produced by the direct oxidation of glucose, which in any case seems more probable than that it is formed by the building back of 2-C compounds. This is also supported by the fact that Müller's[36] gluco-oxydase, which converts glucose to gluconic acid, is widely distributed.

The calculated maximum yields are based on the fact that if the acids are rebuilt from the alcohol or acetic acid formed by the Neuberg reactions, the equation of their formation may be summarised thus:

$$C_6H_{12}O_6 + oxygen \rightarrow 2CO_2 + acid.$$
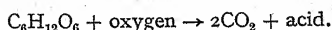
Some of the sugar is almost certain to be converted into other products such as the actual plant body and glycogen besides carbon dioxide and organic acids and, consequently, the very high yields of these acids recorded are most remarkable[1]. They do not amount to a demonstration that the rebuilding from 2-C compounds is impossible, but it is clearly desirable that complete balance sheets recording the fate of all the carbon originally supplied as sugar and calcium carbonate should be obtained under conditions which favour these large yields.

(g) *The fate of the organic acids in fungus cultures*

It is well known from the work of Currie (24), Wehmer (44–46), Bernhauer (3, 4), Molliard (34) and others that the organic acids produced by mould fungi accumulate to maximum concentrations in the culture solutions and then disappear. That circumstance gave rise to the old view that the acids were intermediate products in the respiratory oxidation of sugars to carbon dioxide and water. This view is clearly expressed by Currie (24) and Chrząszcz and Tiukow (21) who give the "equation of respiration" as:

$$\text{Glucose} \rightarrow \text{citric acid} \rightarrow \text{oxalic acid} \rightarrow CO_2.$$
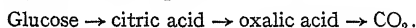
It does provide a rational explanation of the cause of accumulation of the acids and of the significance of their formation. This view, moreover, gives a reason for the fact that the maximum concentration of gluconic acid is attained before the maximum concentration of citric acid, which again is followed by the attainment of the maximum oxalic acid concentration.

The last stages of this process were supposed to proceed as follows (cf. (18, 43)):

$$
\begin{array}{llll}
CH_2.COOH & CH_2.COOH & CH_2.COOH & CH_3.COOH \\
| & | & | & \rightarrow \\
C(OH).COOH \rightarrow & CO \rightarrow & COOH & +CO_2 \\
| & | & & \\
CH_2.COOH & CH_2.COOH & + CH_3.COOH & \\
& + CO_2 & & \\
\text{Citric acid} & \text{Acetonedicarb-} & \text{Malonic +} & \text{Acetic acid} \\
& \text{oxylic acid} & \text{acetic acids} &
\end{array}
$$
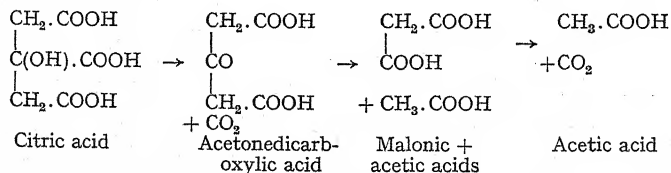
and the acetic acid so formed was supposed to be oxidised via glycollic and glyoxylic acids to oxalic acid, which in turn was oxidised to carbon dioxide and water. Actually the only evidence in favour of this view is (apart from its reasonableness from a purely chemical point of view) contained in the two papers by Challenger and his

---

[1] Allowance was made by Bernhauer, Böckl, and Siebenäuger for the formation of products stored inside the fungus.

co-workers (18, 43). They detected acetone-dicarboxylic acid in cultures of *Aspergillus niger* on ammonium citrate by means of its reaction with benzene-diazonium chloride (Part I, p. 50) which gives mesoxal-aldehyde-bis-phenylhydrazone. 1·3 grm. of this compound were obtained from a culture supplied with 20 grm. of ammonium citrate + 4 grm. citric acid. A culture supplied with 30 grm. of citric acid yielded 3·4 grm. of malonic acid.

Evidently these reactions can take place, but the detection of traces of these products in the cultures does not show that these reactions represent the fate of the greater part of the citric acid which is decomposed by the plant. The views which are held at present by the majority of workers as to the position of citric and other acids in fungus metabolism have been usefully summarised by Bernhauer and Siebenäuger (6). These views are indicated by the reactions given in Table V.

Some explanation of this table is desirable. Oxidation type I is the Neuberg reaction followed by rebuilding of 4-C from 2-C compounds. Oxidation type II includes special oxidations such as the formation of gluconic and other compounds of which the configuration so closely resembles glucose that it seems probable that they are formed from glucose directly. The direct conversion of acetic to oxalic via glycollic acid has been discussed. This change must be regarded as either not occurring or else as accounting for the fate of only a very small fraction of the acetic acid converted into other substances in many if not all mould fungi. It will be noted that the Challenger hypothesis of the fate of the citric acid is accepted by Bernhauer. The various acids are assumed to pass through a cycle of reactions which appear to the present writer to give no reasonable explanation of the occurrence of maxima in their concentrations at definite times. The cycles of changes appear to have little if any biological significance, though Chrząszcz and Tiukow (20, 21) have suggested that citric acid is accumulated in *Penicillium* cultures as an alternative storage product to starch.

It is possible to obtain a direct test of the validity of some of the reactions propounded in Table V. It will be noted that except for the production of acetone, aldehyde, and alcohol, all the acids are supposed to give rise to other acids or to carbon dioxide. Furthermore all the carbon dioxide formed is split off from carboxyl groups of the acids. So the net decrease in titratable acidity is due to the splitting up of the carboxyl groups thus: $R.COOH \rightarrow R.H + CO_2$. Therefore the disappearance of each equivalent of titratable acid

from the culture must be associated with the formation of at least one molecule of carbon dioxide, if the scheme of reactions in Table V is valid.

TABLE V

Interrelations of products of fungus metabolism
(Bernhauer and Siebenäuger)

```
Saccharic acid
    ↖
    │        Gluconic acid        "Kojic acid"        TYPE II
    │             ↖              ↗
    │                Sugar
    └ ─ ─ →  └ ─ ─ →  Methyl glyoxal                  TYPE I
                         ↓
                      Pyruvic acid
                         ↓
                      Alcohol
                         ↓
            ┌─────────→ Acetic acid
            │          ↙          ↘
            │   Succinic acid        Glycollic acid
            │        ↓                    ↓
            │   Fumaric acid         Glyoxylic acid
            │        ↓                    ↓
            │   Malic acid            Oxalic acid
            │     ↙      ↘
            │ Oxalacetic acid    Citric acid
            │     ↙                   ↓
            │ Acetic + oxalic acids   Acetonedicarboxylic acid
            │                         ↙          ↘
            └──── Acetic + malonic acids    Acetone + CO₂
                       ↓
               Acetic acid + CO₂
```
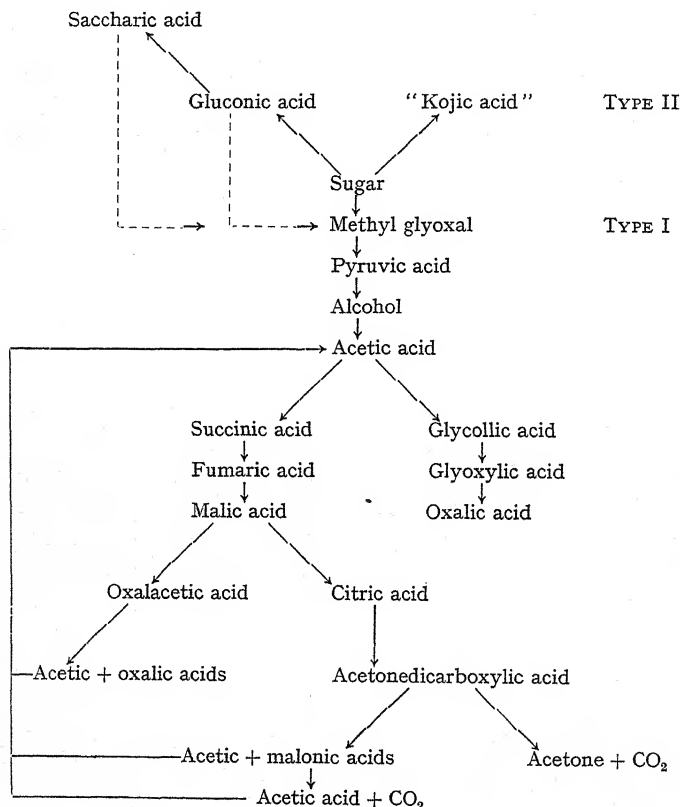
The writer is indebted to Mr La Touche for agreeing to the publication here of some of the results of our joint work carried out to test this point. It may be said at once that our findings are totally opposed to the views of the fate of the acids which are incorporated in Table V. They show that the disappearance of a number of acids (citric, malic, glycollic and oxalic were investigated) is associated with no extra output of CO_2 as would be required by the decarboxylation theory.

The method of experiment somewhat resembled that of Kosinski (27). A continuous record of the rate of CO_2 output of cultures of *Aspergillus niger* grown on nutrient solutions containing sugar was obtained by the Pfeffer-Pettenkofer method. The nutrient solution was then run off and replaced by distilled water under sterile conditions. The effect of this, as Kosinski showed, is to cause the rate of carbon dioxide output to decrease roughly logarithmically to a low value which is maintained constant for some days presumably at the cost of the carbon compounds stored as reserves in the mycelium. Our work shows that the stores of glycogen slowly decrease during this
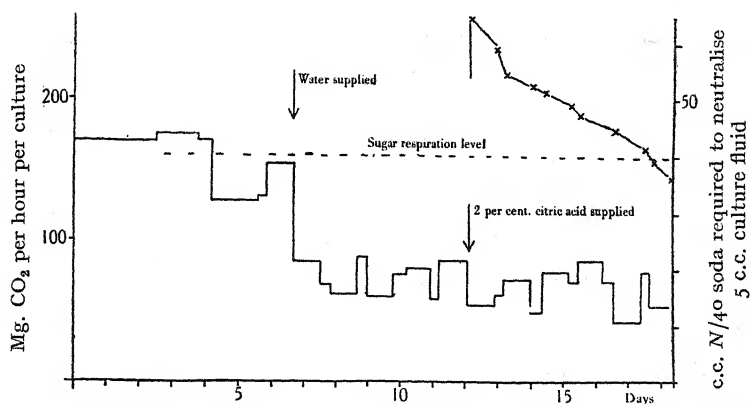
Fig. 3. The effect on the rate of CO_2 output of an *Aspergillus niger* culture of change from 5 per cent. glucose + Czapek's nutrient solution, to water (first arrow) and then to approximately 2 per cent. citric acid (second arrow). During the last phase when the fungus mat is supplied with citric acid, the change in titratable acidity is recorded thus ($\times$—$\times$). (Bennet-Clark and La Touche, unpubl.)

"starvation phase" of the respiration. The source of this steady carbon dioxide output is therefore presumably the reaction series:

$$\text{Glycogen} \rightarrow \text{sugar} \rightarrow CO_2 + H_2O$$
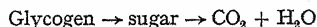
and the steady rate is maintained by the rate of the hydrolysis of glycogen. If the sugar concentration increases as a result of the artificial addition of sugar the rate of carbon dioxide output is increased.

Citric acid solution (and in other experiments other acids) was then supplied to the culture in place of the distilled water which was run off. The graphical record of a typical experiment of this nature is recorded in Fig. 3, and shows that this addition of citric acid has no effect whatever on the rate of carbon dioxide output.

One must therefore conclude that all the carbon dioxide formed, even after citric acid has been supplied, is produced from glycogen as indicated above and that none is formed from the added acid.

It is found that the citric acid which is added disappears very rapidly at first, but with gradually decreasing rapidity. The rate of citric acid disappearance in the case of this particular experiment is recorded in Fig. 4. The rate of disappearance of acid is expressed in
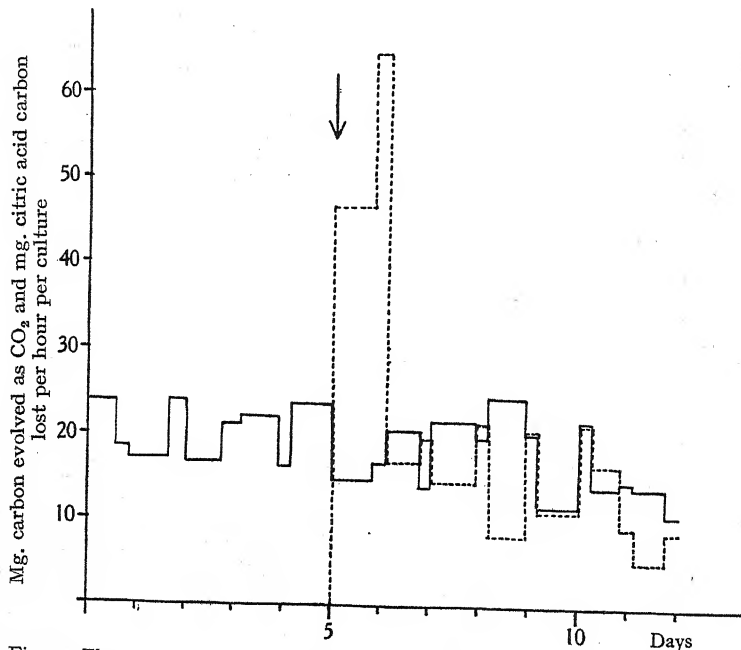
Fig. 4. The rate of loss of carbon from a culture of *Aspergillus niger*, first supplied with water only, and then with roughly 2 per cent. citric acid. The rate at which carbon is lost from the form of citric acid is shown by the dotted record. CO_2-C output is shown by the continuous record. Citric acid supplied at time indicated by the arrow.

terms of numbers of mg. of carbon disappearing from the form of citric acid per hour. The quantities of citric acid present were determined by titration with soda. It was shown that the disappearance of citric acid was not associated with the accumulation of other acids in the culture solution. The quantities of citric acid determined by titration agreed reasonably closely with the quantities as determined by conversion into pentabromacetone, and also with the carbon content of the culture solution determined by the wet combustion method developed by Birkinshaw and Raistrick[10].

Fig. 4 records the rate of disappearance of citric acid carbon and also the rate of output of carbon dioxide carbon. It will be noted that not only does the supply of citric acid to the "starving" fungus cause no *increase* in the rate of carbon loss (as CO_2), but the *total* rate of carbon loss is less than half the rate of citric acid carbon loss during a period of about 48 hours.

This result shows that the loss of titratable acidity is certainly not brought about by the decarboxylation of the citric acid or of any acid derived from it by hydrolysis or splitting.

The scheme of reactions put forward by Bernhauer and Siebenäuger indicates that eventually all the carbon of the citric acid is evolved as carbon dioxide. This obviously does not take place, since the quantity of acid-carbon lost so greatly exceeds the quantity of CO_2-C evolved. It seems reasonable to suppose that all the carbon dioxide evolved is derived from the glycogen reserves in the fungus, since the results show that the rate of carbon dioxide output is the same when no external source of carbon supply is present as it is when citric acid is supplied. But even if this assumption were not regarded as reasonable the *total* CO_2-C loss is too small to account for the observed decrease in titratable acidity by the scheme given in Table V.

The loss of titratable organic acid may be brought about by the following reactions:

(i) Formation of esters or anhydrides: $R.COOH + H.X \rightarrow R.CO.X + H_2O$.
(ii) Formation of amides: $R.COOH + NH_3 \rightarrow R.CO.NH_2 + H_2O$.
(iii) Formation of salts: $R.COOH + NH_4OH \rightarrow R.COONH_4 + H_2O$.
(iv) Decarboxylation: $R.COOH \rightarrow R.H + CO_2$.
(v) Reduction to aldehydes: $R.COOH + 2H \rightarrow R.CH = O + H_2O$.

Regarding reaction (i), readily hydrolysable esters or anhydrides are not present in the fungus in sufficient quantity. Reactions (ii) and (iii) cannot account for the disappearance of the acid, since neither the body of the solution nor the mycelium contain appreciable quantities of amides or salts of either citric or other organic acids[1]. Reaction (iv), which is universally assumed to be the cause of the disappearance of citric and other acids, is not the true cause of this disappearance, since it is now shown that no extra carbon dioxide is evolved during the phase when acid is disappearing. One is therefore left with reaction (v) as the only available alternative. It is not unreasonable to suppose that such reduction to aldehydes occurs, since it is well known that the glycogen content increases when fungus mycelia are

[1] The *total* nitrogen content of the fungus is moreover too small.

supplied with organic acids as the sole source of carbon. Investigators of fungus metabolism have not committed themselves as to the chemical nature of this process, but it is probable that such reactions as

(i) Citric or malic acid → oxalacetic acid → aldehyde + $2CO_2$,

(ii) Aldehyde + oxygen → carbohydrate,

would be assumed to occur, as Wolf (48) has assumed in the case of the succulent plants.

Since the observed carbon dioxide output derived from the disappearing acid is zero, this explanation seems less probable than the alternative that the acid is reduced to a hydroxy-aldehyde, which is polymerised (or in some similar way converted) into carbohydrate.

We have also shown that the disappearance of a variety of other organic acids from fungus cultures is associated with no extra production of carbon dioxide. Of these one of the more interesting is glycollic acid. Our results agree with those of Raistrick and Clark (38) and Bernhauer and Scheuer (7) in that we found no oxalic acid in cultures supplied with glycollic acid. Rapid disappearance of the glycollic acid (as determined by titration) occurred and it follows that it must be converted into either methyl or ethyl alcohol, formic or acetic aldehyde, glycollic aldehyde or glyoxal. Direct analysis showed that volatile carbon compounds (other than CO_2) were present in negligibly small traces as compared with the quantity of glycollic acid which disappeared, and this, combined with the fact that the disappearance of the acid is associated with no extra carbon dioxide output, indicates very clearly that either the acid is converted to glycollic aldehyde or glyoxal, or else that it is converted by the Thunberg reaction into some other acid such as malic acid which is reduced to an aldehyde. Such hydroxy-aldehydes have not been detected and it seems probable that they may be immediately converted into carbohydrate.

It has not been found possible yet to obtain direct evidence that a gain of glycogen or other carbohydrate occurs at the expense of the disappearing acid, as the amount of gain expected from the observed extent of the acid loss is only a very small percentage of the glycogen content, and the sampling and analytical errors are therefore as large as the expected gains in glycogen content.

In concluding this section it may be pointed out that the evidence does not compel one to suppose that citric acid, for example, is reduced to its own aldehyde. It might be hydrolysed yielding malic and glycollic acids, which might be reduced to aldehydes.

It should be pointed out that similar conclusions regarding the fate of the organic acids in succulent plants have already been drawn[2] on the basis of somewhat similar evidence.

(h) Conclusion

The balance of evidence favours the view that sugar breakdown follows the Neuberg reactions in yeast and mould fungi, and that the 2-C compounds are resynthesised into the 4-C acids and citric acid. This resynthesis has been shown beyond doubt to take place. It should be pointed out, however, that Raistrick and his co-workers as recently as 1931[39] have summarised their views on the sugar metabolism of mould fungi in these words: "It appears that the first stage (of glucose breakdown) is a Cannizaro reaction involving the production from two molecules of glucose, of one molecule of mannitol and one molecule of gluconic acid. Depending then on whether the mould prefers for growth an acid or a neutral medium either mannitol or gluconic acid (of course in some cases both) is destroyed. There is ample evidence to support this view."

The ample evidence is not described. It is pointed out that white Aspergilli produce a neutral reaction of the culture medium and that they produce mannitol the maximum yield of which never exceeds 50 per cent. of the glucose used as demanded by the statement above. On the other hand the yields of gluconic acid produced by coloured Aspergilli and Penicillia frequently greatly exceed 50 per cent.; this is not mentioned nor are the high yields of other acids explained. A difficulty is mentioned but not explained in the fact that glucose on reduction yields sorbitol and not mannitol.

The position of the organic acids may be represented by the sequence of reactions, scheme I, according to the views of Chrząszcz, Tiukow and Bernhauer. The present writer, for reasons detailed in the last section, regards the closely similar scheme II as more probable.

$$
\begin{array}{ll}
\text{I} & \text{II} \\
\text{Carbohydrate} & \text{Carbohydrate} \leftarrow \text{Carbohydrate} \\
\downarrow & \downarrow \qquad\qquad\qquad \uparrow \\
\text{Methyl-glyoxal} & \text{Methyl-glyoxal} \\
\downarrow & \downarrow \\
\text{Pyruvic acid} & \text{Pyruvic acid} \\
\downarrow & \downarrow \\
\text{CO}_2 + \text{2-C compounds} & \text{CO}_2 + \text{2-C compounds} \\
\downarrow & \downarrow \\
\text{4-C acids and} \leftarrow & \text{4-C acids and} \rightarrow \text{Hydroxy-} \\
\text{citric acid} & \text{citric acid} \qquad \text{aldehydes} \\
\downarrow & \\
\text{CO}_2 + \text{2-C compounds} &
\end{array}
$$
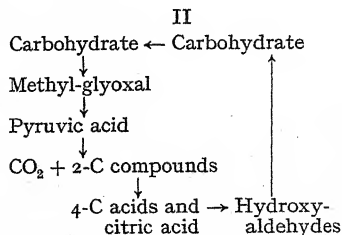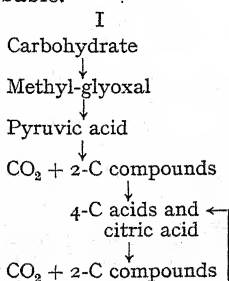
Scheme I fails to explain the accumulation and final disappearance of citric acid, which can be explained by the second scheme if it be assumed that the velocity of formation exceeds that of reconversion of the acid. These views based on the Neuberg reactions fail to explain the rôle of gluconic acid or oxalic acid formation, and the peculiar effects of nitrogen compounds on the citric and gluconic acid production in Aspergilli, and, so, though they may form useful working hypotheses for the guidance of further research, one cannot regard these views as more than a first approach towards the elucidation of the part played in fungus metabolism by the plant acids.

On the other hand scheme II, which may be summarised thus:

$$\text{Sugars} \rightleftharpoons \text{plant acids} + \text{energy}$$
$$\downarrow$$
$$CO_2$$
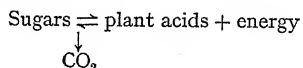
involves a very large energy change, and it is by no means clear either how the energy for the postulated resynthesis of carbohydrate from acid is supplied, or what the significance of the change is. It must be pointed out that one requires very convincing evidence of the occurrence of such remarkable changes as the conversion of the plant acids into carbohydrates. The evidence does seem to show that in such diverse groups as the mould fungi and the succulent plants the organic acids are caused to disappear by processes other than decarboxylation. Reduction to aldehydes seems the most likely cause of their disappearance, and conversion of such hydroxy-aldehydes to carbohydrates is at least possible.

II. The formation of the plant acids in protein metabolism

Kostytchev and more recently Ruhland and Wetzel have been the chief supporters of the view that the plant acids are formed in the course of protein metabolism rather than as essential products in the respiratory breakdown of carbohydrates.

Kostytchev [28, 29] evidently originally considered, and Ruhland and Wetzel [40, 41] apparently still consider, that the plant acids are formed by oxidation of proteins or products of protein hydrolysis, and that they are not formed by the oxidation of carbohydrate. This view was originally propounded in order to bring the formation of the plant acids into line with the supposed breakdown of sugars according to the Neuberg scheme. Direct evidence in favour of the protein origin of the plant's acids is almost unobtainable, but four lines of evidence are held by these workers to give general support to this view.

(i) The most quoted experimental work bearing on this subject is Ehrlich's work on the conversion of glutamic acid to succinic acid by yeast[25]. As pointed out earlier (p. 208) he showed that the amount of succinic acid produced is increased by extra supplies of glutamic acid, but it must be borne in mind that this increased yield of succinic acid is not obtained when other nitrogen supplies are also present (see Table II, p. 208). This conversion of glutamic acid is consequently associated with nitrogen hunger, and one cannot be certain that succinic acid is normally formed by this process. Ehrlich supposed that the conversion of glutamic acid proceeded thus:

$$COOH.CH_2.CH_2.CHNH_2.COOH \rightarrow COOH.CH_2.CH_2.CHOH.COOH + NH_3$$

followed by oxidation of the hydroxy-glutaric acid to succinic acid. It seems likely, however, that this deamination is an oxidative process as it has been shown that many amides and amino acids are more rapidly deaminated in the presence of hydrogen acceptors. Neubauer and Frommherz[37] have also shown that yeast converts phenyl-glycine ($C_6H_5.CHNH_2.COOH$) into phenyl-glyoxylic acid ($C_6H_5.CO.COOH$), and it is now generally thought that the conversion of glutamic or other amino acids follows a similar course, thus:

$$COOH.CH_2.CH_2.CHNH_2.COOH + O \rightarrow COOH.CH_2.CH_2.CO.COOH + NH_3$$
$$COOH.CH_2.CH_2.CO.COOH \rightarrow COOH.CH_2.CH_2.CH = O + CO_2$$
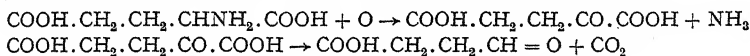
followed by oxidation of the aldehyde to succinic acid.

Kostytchev and Frey[29] suggest that the malic acid which they detected in fermenting yeast cultures similarly is formed by the oxidative deamination of hydroxy-glutamic acid. This demonstration by Ehrlich that in the presence of sugar[1] and absence of other nitrogen supplies glutamic acid reacts as described does not provide reasons for presuming that succinic acid formation by plants normally proceeds by this course rather than by oxidative synthesis from 2-C compounds. Both sources may contribute to the normal formation of the acid.

(ii) The close similarity of structure between malic acid and asparagine and the inter-connection of the latter with the protein metabolism of plants certainly suggests that the malic acid metabolism and protein metabolism are connected with each other, but this provides no evidence that the carbon skeleton of the asparagine is derived from protein sources. Mothes'[35] results suggest that the carbon skeleton is formed from carbohydrate in a manner similar to that occurring in succulent plants. He showed that feeding bean

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[1] It is not usually emphasised that succinic acid is not formed when glutamic acid only without sugar is present.

seedlings with ammonium salts greatly increases the amide content of bean seedlings which seems to favour the view that the carbon part of the amides arise from carbohydrate and the nitrogen from any available source of ammonia; from external supplies as in this experiment or from internal supplies made available if protein breakdown occurs. The fact that bean seedlings kept in darkness accumulate asparagine normally at the expense of protein but that in the presence of narcotics this asparagine formation is prevented and ammonia accumulates instead, is also in general agreement with this view. Malic acid formation from carbohydrate is inhibited by narcotics, the supposed reason being that anabolic processes are involved in its formation, and thus asparagine formation would naturally be prevented by narcotics.

(iii) Ruhland and Wetzel point out that the disappearance of amino acids and proteins either in normal metabolism or under special experimental conditions is associated with the formation either of amides or ammonium salts. They group plants under the two headings "amide plants" and "ammonium plants." To the latter group belong *Rheum, Begonia,* and *Aspergillus.* As was shown in Part I, the disappearance of amino acids from *Rheum* is associated with the accumulation of ammonium malate in the leaf stalks. Disappearance of amino acids from cultures of *Aspergillus* supplied with peptone as the sole source of carbon is similarly associated with the accumulation of ammonium oxalate in the culture [11]. *Begonia* is also rich in ammonium oxalate, which, it is suggested, is formed from amino acids.

These observations suggest that the carbon residues formed by the deamination of amino acids may in part be converted into malic acid and oxalic acid. However, one can hardly suppose that the great variety of different carbon residues are all or almost all converted into these two simple compounds during normal metabolism. It seems more probable to the writer that these nitrogen-free residues are utilised in the many complex syntheses which the plant conducts, and that the ammonia set free reacts with the malic and oxalic acid formed from carbohydrate in respiration yielding ammonium salts or asparagine.

The evidence of the type cited in this section certainly does not run counter to the views that malic and oxalic acids are formed from carbohydrate in respiration. No one, however, would suggest that they are never formed from protein sources, but no details are yet available regarding the chemical mechanism of the conversion of

amino acids to malic and oxalic acids except in the case of glutamic acid.

(iv) Kostytchev has stated that it is unthinkable that the branched chain structure of citric acid could arise from carbohydrate as an intermediate product in respiration. The difficulty is removed if one does not regard it as a product of sugar respiration, but instead regards it as formed from *iso*-leucine:

$$\begin{array}{c} CH_3 \\ >CH.CHNH_2.COOH \\ CH_3.CH_2 \end{array} \rightarrow \begin{array}{c} COOH \\ >C(OH).CH_2.COOH \\ COOH.CH_2 \end{array}$$
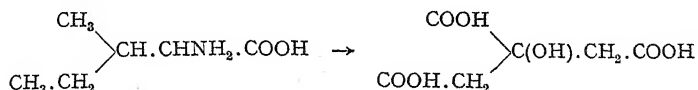
He admits, however, that carbohydrate is the ultimate source from which the greater part of the citric acid is formed in mould fungi. But he regards the acid formed as a by-product of protein formation and not as an intermediate product of respiration. The evidence for this view is that Kostytchev and Tschesnokow[30] found that acid accumulation only commenced in *Aspergillus niger* after the nitrogen supplies in the cultures had been used up. This result is held to show that the carbon units available for protein synthesis are used for this purpose while nitrogen supplies are available, but that after all the nitrogen has been used up, these carbon residues can no longer be converted into protein and are consequently converted into organic acids (citric and oxalic acids in the case of *Aspergillus niger*). Further evidence in favour of this interpretation is not available at present. The actual facts quoted cannot be accepted as having a very general significance in view of the findings of Molliard[34] and Bernhauer[3] which have been discussed already in section (c). Chrząszcz and Tiukow[23] also show that acid accumulation occurs in the presence of abundant supplies of nitrogenous substances.

Kostytchev complains[30] of misquotation, and states that he never considered that the plant acids could not arise from carbohydrate. It is clear, however, that he preferred to consider that they arose from protein sources unless the quantity formed (as in mould fungi) is far too large for this to be possible. For example, he considers that the malic acid produced by yeast is formed by the oxidative deamination of hydroxy-glutamic acid[29]. This point of view is summarised in Kostytchev's statement ([28], *original edition*): "Die Oxydation der gewohnlichen Pflanzensäuren stellt einen Vorgang dar, der mehr Ähnlichkeit mit der Eiweissathmung als mit der Zuckerathmung hat." Kostytchev[30] later emphasised that this

statement refers to the oxidation and not to the formation of the plant acids, which he admits may be formed from carbohydrate. If that is so, it is not easy to understand what the term "Eiweissathmung" means: when these provisions are admitted "Eiweiss-" and "Zuckerathmung" become almost identical.

(v) In conclusion Benecke's view (1) should also be borne in mind that the production of acids is associated with the formation of protein from nitrates. It was supposed that the free base liberated on reduction of nitrates to organic nitrogen compounds was neutralised by the plant acids, and these were supposed to be produced for the purpose of effecting this neutralisation. The recognition of this possibly useful effect which is a consequence of their production does not throw light on the mechanism of their formation.

Ruhland and Wetzel (40) hold the somewhat similar view that the function of the acids is the depoisoning of ammonia formed in active protein respiration. One might with equal justification put forward the view that the ammonia is produced from protein in order to depoison the acids formed in carbohydrate breakdown, indeed Butkewitch's results (11) are more favourable to this latter view. The real significance of the formation of the plant acids will only become known when the manner in which the energy set free in cell oxidations is more clearly understood.

III. General conclusion and summary

General pronouncements on the significance of the plant acids are at present probably of little value. It is, however, worth while to summarise the more important reactions in which these substances are known to take part. The reactions given in Table VI indicate the possibility of occurrence of a variety of changes which have been observed in different organisms.

It should be remembered that it is in general impossible to obtain evidence that a reaction does not take place in any given plant tissue. In fact the occurrence of any reaction can usually be detected only under conditions peculiar to a particular tissue or a particular experimental treatment which cause this reaction to predominate.

Thus, for example, the conversion of glutamic to succinic acid occurs when fermenting yeast is supplied with glutamic acid in the complete absence of other nitrogen supplies. This reaction is generally assumed to occur also in other tissues as well as yeast and also under quite different conditions. But proof of this common supposition is usually impossible, since succinic and the other 4-C acids are also

formed from 2-C compounds which may be derived from carbo-
hydrate or other sources, and it is impossible to tell which process is
responsible for any observed succinic acid production.

TABLE VI

The metabolic behaviour of the plant acids
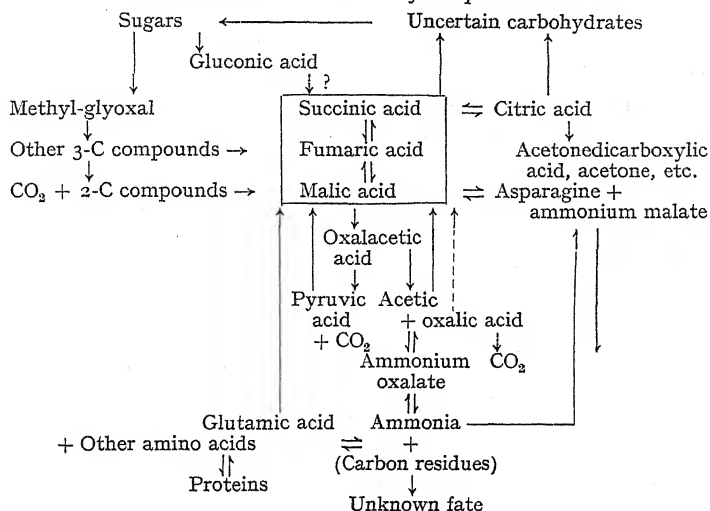
Our knowledge of plant acid metabolism is therefore best sum-
marised by including all the reactions which have been shown to
occur in living tissues, since it is usually impossible to show that such
reactions do not occur in any given tissue, but one must emphasise
that Table VI gives no information as to the relative rates or velocity
constants of the several reactions or of the resultant effects on the
concentrations of the reactants. These are very different in different
tissues and are, moreover, much affected by external conditions.

Some of these reactions (see Table VI) call for special comment.
It is at present impossible to decide if alternative modes of sugar
breakdown to the Neuberg reactions occur. The gluconic acid meta-
bolism of fungi presents some difficulty, since it is clear that large
yields of gluconic acid can be obtained from fungi which produce
acetaldehyde and alcohol under anaerobic conditions. Such fungi
possess both the zymase complex of enzymes and gluco-oxidase.
Regarding the conversion of the plant acids into carbohydrate, the
evidence that this process occurs and that it involves reduction of
the acids to aldehydes has been dealt with at length in the sections
on mould fungi and succulent plants.

Evidence exists that oxalic acid is also reduced in plant tissues and it is possible that it is also oxidised to carbon dioxide, but it is not possible to obtain direct evidence of the occurrence of this latter process, though it appears plausible on general chemical grounds. No speculations as to the fates of the nitrogen-free carbon residues of the amino acids have been included in the scheme of reactions. They may in part be oxidised and may thus provide alternative sources of the plant acids. Three alternative fates for citric acid are indicated: two involve reduction of the carboxyl groups, the third (oxidation with CO_2 evolution) hardly occurs under strict aerobic conditions.

There is at present no evidence to show how the energy necessary to effect the postulated conversion of the plant acids into carbohydrates is supplied, and one must presume that it is made available either by oxidation of part of the acid or of some other substance. One regards this resynthesis in the same light as the "oxidative anabolism" of Blackman or the oxidative resynthesis of glycogen from lactic acid in muscle, or from alcohol in yeast.

The true significance of the acid metabolism of plants may perhaps lie close to Kostytchev's later point of view that these acids are the building stones from which some of the complex plant products originate. It has been pointed out [43] that succinic-dialdehyde and acetone-dicarboxylic acid, for example, are the ingredients of Robinson's synthesis of tropinone, the parent substance of a number of alkaloids. The plant acids are possibly convertible into amino acids and proteins, complex products such as the alkaloids, and by reduction into the parent substances of the fats and also into carbohydrates. Thus they may form some essential links in the processes by which the energy of respiration is transferred to the varied synthetic activities of the plant. It is certainly clear that they are by no means waste products of metabolism.

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FLORAL ANATOMY AND ITS MORPHO-LOGICAL INTERPRETATION[1]

By AGNES ARBER

IN a subject whose origin dates back so far as that of botanical morphology, certain assumptions are liable, through long usage, to become part of the recognised currency of thought, although most of those who employ them have never made any effort to test their validity. One such postulate, which has been widely accepted, is that the facts of vascular anatomy serve as a guide to phylogenetic history. It may be worth while to attempt some scrutiny of this assumption, and, for the sake of clearness, to put the problem in the form of the bald question—*whether the use of anatomical evidence in phylogenetic morphology can ever be justified?* It is evident that such a question may be understood in a variety of senses; if it is viewed in the broadest and least detailed aspect, it demands, I think, an affirmative answer. For there is evidence that the *general* nature of the vascular scheme may have a certain systematic value; in other words, some weight may be attributed to it as an indicator of trends in race history. There is no doubt, for instance, that the work of Robert Brown (9) and Darwin (12) on the anatomy of orchids, showed that the flower in this family is more conformable to the general Monocotyledonous type than was formerly supposed. The value of the anatomical method—in the broad sense—comes out clearly, also, in Ethel Sargant's comparative investigation of the seedlings of the Liliaceae and their mode of anatomical transition from root to stem (20). But when from the general symmetry of the seedling, considered as a whole, this author turns to the individual bundles, her argument appears to me to lose its cogency. She held, for instance, that the existence of two bundles in the cotyledon of *Anemarrhena* pointed to its derivation from the fusion of two ancestral seed-leaves. The assumption on which this opinion was based was that the vascular system might be expected still to reveal an ancestral duality, after the external form had ceased to show any sign of this primitively dual character. But is this postulate justified? The crux lies in whether we are to consider it proven that the vascular bundles are

more "conservative" than the external form, so that vestigial organs may be represented by their bundles, when all external trace of these organs has disappeared. This idea seems to have received its first definite formulation from Henslow(17), and since his day it has been accepted, without criticism, by more than one writer in this country. A description of the hypothetical "solid" type of carpel may be taken as an extreme example. We are told that in "its most reduced form it consists merely of a fibro-vascular cord with but few lateral veins and reticulations connecting it with its neighbours, which have in the course of their own expansion, so to speak, engulfed it"(21). This statement involves the belief that a carpellary leaf may be reduced to its vascular bundle *alone*—this bundle being embedded in tissue belonging to other carpellary leaves; but we have no right to accept this view unless it can be shown that there is *evidence* for such survival of the vascular system of an organ of which no external trace exists. To see whether any such evidence can be found, I will now consider those examples of reduced leaf structures, foliar and floral, which I have myself met with in the course of anatomical work.

In the grass *Luziola Spruceana* Benth., the outer empty glumes are rudimentary and nearly always non-vascular, but a strand corresponding to each glume usually occurs in the axis, though these strands generally die out before the glume is reached ((2), Fig. 3, I_1 and I_2, K_1 and K_2, p. 397). Moreover, both in other grasses and elsewhere, I have met with instances of further reduction, in which the vascular supply has vanished altogether. For example, the ridge at the base of the ear in cultivated barley (*Hordeum vulgare* L.) is clearly a reduced leaf, for it may bear a bud in its axil, but I find that it has no vascular tissue, though it has a considerable development of mesophyll ((3), Fig. 1, *A, B, C*, p. 508). Again, the inflorescence branches of *Luziola Spruceana* Benth. occur in the axils of similar rudimentary leaves which are wholly non-vascular ((2), Fig. 1, p. 392). Corresponding examples may be found among floral leaves. In *Corydalis nobilis* Pers. and *C. lutea* (L.) DC., there is a single pair of small sepals with a normal vascular supply. In *C. bulbosa* Reichb., on the other hand, the equivalent pair of sepals is extremely reduced, and there is no vascular supply for them, either in the receptacle or in the free part of the sepal ((4), p. 342).

The facts about vestigial structures cited in the preceding paragraph all relate to leaves or sepals; they may be supplemented by some observations on the androecium—a part of the flower which is particularly favourable for such study, since members, which

botanists agree in regarding as abortive stamens, occur more fre-
quently than rudiments of other parts of the flower. The condition
of the rudimentary fifth stamen in *Antirrhinum* and *Digitalis* is
significant for our argument. The posterior stamen of *Antirrhinum
majus* L. is represented by a short antherless filament. I have traced
the structure of this filament by means of serial sections in five
flower-buds, and in each case I found a bundle which died out before
the apex of the filament was reached. In a series of sections
through the tip of an inflorescence of *Digitalis purpurea* L., I found
a rudiment of the posterior stamen in five successive flowers[1]. It was
extremely small—much less developed than in *Antirrhinum*—and
was most clearly visible in a bud which did not exceed 2 mm. in
diameter ((6), Fig. 2, *C*); in older flowers it was not recognisable. The
free existence of the rudimentary filament was minimal and there
was no corresponding bundle. Here reduction had gone further than
in the flower of *Antirrhinum majus*, and the stamen, though still
existing in a rudimentary form, had completely lost its vascular
supply. The fact that certain floral members may contain no vascular
tissue is, indeed, not new; instances have been known since the days
of van Tieghem (26).

The descriptions of the anatomy of rudimentary organs in the
literature, and the examples which I have enumerated above, all
lead to the same conclusion: that a rudimentary external form is
found to correspond to a vascular system which is equally, or even
more, rudimentary; indeed an organ, which retains some trace of
its normal external form, may yet show a complete lack of vascular
tissue. It thus becomes clear that we have no alternative but to
discard the doctrine of the conservatism of the vascular bundles.

The genus *Hypecoum* supplies an example of misinterpretation of
an androecium through adherence to the erroneous idea of vascular
survival. The anatomy in this genus has been cited as revealing that
the ancestral form must have possessed two more stamens than its
modern descendants, since the inner stamens, though otherwise normal,
may have broad filaments each supplied by two bundles instead of
by the single strand typical for androecium members. Hildebrand
and others have concluded, from the existence of the two bundles,
that the inner stamens of *Hypecoum* each represent a pair, ancestrally
free, but now in a state of fusion. I have recently shown, however,
that the existence of these paired bundles is an accidental by-product

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[1] The degree of development of this rudiment probably varies in different
material.

of a particular mode of bundle-branching, associated with the superposition of a stamen to a petal(5). The two-bundled character can thus be explained on simple mechanical grounds; there is no need to invoke anything so romantic as an hypothetical ancestor in order to understand it. The androecium of the Primulaceae provides another, and perhaps even more striking example, in which the belief in vascular survival has been responsible for an artificial and erroneous interpretation. In this family van Tieghem(25), Henslow(18), and their followers ((22) etc.), have described bundles given off internally from the calyx midribs, on the same radius; they regard these bundles as representing the absent whorl of stamens. If this were indeed so, it would be a weighty piece of evidence for the continued production of bundles after the organs which they formerly supplied have ceased to exist. I find, however, on examining serial sections of the flowers of *Primula vulgaris* Huds. and *Anagallis arvensis* L., with this question in view, that, though five bundles superposed to the sepal bundles certainly exist in the corolla tube, *there is no reason to interpret them as stamen bundles*. They appear to be merely laterals belonging to adjacent petals, which exist in a state of fusion in the corolla tube, but separate with the separation of the corolla lobes.

There seems, indeed, to be no escape from the conclusion that there is a complete absence of positive evidence for the vestigial survival of vascular tissue after the organ which it supplied has ceased to exist. After I had come to this result, I found that it had been anticipated, some thirty-five years ago, by Grélot in his memoir on the floral anatomy of the Gamopetalae[1](15). His conclusion, drawn from a wide series of observations, is that "lorsqu'il y a avortement partiel d'une pièce, on trouve ordinairement un ou plusieurs faisceaux rudimentaires qui lui sont destinés; *lorsqu'il y a avortement total, il n'existe jamais de faisceaux.*" Elsewhere he adds, "*en règle générale, un faisceau floral est toujours destiné à un organe représenté extérieurement,* si petit que celui-ci puisse être." It follows from these conclusions that the phylogenetic speculations which have been based on the alleged "conservatism" of the vascular strands, can no longer be accepted, and that we must cease to treat morphological interpretation as a puzzle-game elaborated by Nature, towards the solution of which she has thoughtfully provided neat little anatomical clues.

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[1] This memoir, despite its early date, is an admirable example of the application of microtome methods to floral anatomy. The introduction and the second part, which contain some seventy pages of general discussion, deserve more attention than they seem to have received from students of the flower.

The game of guesswork reconstruction of ancestral forms, with its anatomical code, which varies from player to player, seems to have arisen out of the habit of isolating structural details and dealing with them, as it were, *in vacuo*. It is this habit which has led, for instance, to the misconception of the nature of the carpel which has found expression in the "polymorphic" hypothesis. On the theory of the gynaeceum developed by de Candolle[11] and Robert Brown [8, 9, 10], the carpel is a leaf member in which the region in the neighbourhood of the margin is ovuliferous. There is a tendency to-day to overlook the consequences of this shifting of the major activity of the leaf from midrib to margins, in correlation with the change from the vegetative to the sporogenous phase. These consequences were, however, clearly recognised by earlier workers. More than a hundred years ago, Robert Brown wrote that "in the Ovarium...the vascularity, compared with that of the Leaf, is in general rather modified than diminished; the principal vessels occupying the margins or lines of production, and giving off branches toward the axis [midrib], whose vascularity is frequently reduced"[8]. He also suggested that the fundamental type of carpel possessed two lateral stigmas, each derived from the upward continuation of one of the ovuliferous margins; these marginal stigmas might remain single, or they might fuse to form what was apparently one organ[10]. Henslow adopted Brown's idea of the importance of the carpellary margins, and referred to "a form of hypertrophy in the edges of the carpellary leaves, a condition of things widely different from the usually thin and more or less impoverished margins of true leaves"[17]. Grélot, again, pointed out in 1897[15] that the median bundles of carpels "n'ont qu'un rôle très effacé," while the placental or marginal bundles are highly developed. This point has recently been re-emphasised by Dr Hamshaw Thomas, who gives a striking series of figures to show the great importance of the marginal veins, and the laterals derived from them, in the follicles of the Ranunculaceae. He regards the "marginal" veins as the midribs of what he believes to have been, originally, lateral segments of a palmate sporophyll[23], a view which does not seem to differ essentially from that of Prof. Eames, who holds that the carpel is fundamentally a three-trace member[13]. This predominance of the marginal regions in the carpel as compared with the foliage leaf—like many other divergences in organic structure— is not an absolute change, but merely *a variation in relative emphasis*; it is foreshadowed in some foliage leaves in which the laterals are much more conspicuous than the median vein (e.g. many of the

Crocoideae and *Astelia* (1), Fig. xcv, p. 120, and Fig. lv, p. 77). Perianth members, moreover, often show a type of venation intermediate between that of vegetative leaves and carpels (15). It is clear, then, that when the carpel is envisaged on broad lines in comparison with other foliar structures, the predominance of the marginal veins presents no difficulty. Their misinterpretation, on the "polymorphic" view, as distinct carpels, is due to a failure to realise the relation of carpel veining to other types of foliar nervation.

Carpel morphology is not only deeply affected by the revolutionary transition from the vegetative to the sporogenous phase of existence, but also by a second factor—the close relation in which the gynaeceum stands to the shoot apex. It is necessary to bear in mind, in any explanation of the gynaeceum, that the carpels not only are, but presumably always have been, the *ultimate* members borne by a shoot of limited growth—an obvious fact which has hitherto been strangely neglected. Odd anatomical adjustments, of which we ought to know more, take place at the tips of shoots of limited growth. For instance, I have shown by a study of the apices of inflorescence axes in the Fumariaceae, that strands which would naturally be axial, may be diverted as extra-vascular tissue into the uppermost lateral buds (e.g. *Corydalis*, (4), Fig. 2, p. 321, and Fig. 5, p. 327, and *Fumaria*, (4), Fig. 9, p. 344). The study of the apical peculiarities of various types of shoot showing limited growth offers a field which up to the present has been insufficiently explored; I believe that it may be one of the most hopeful directions in which to expect light on floral morphology, for it should illuminate such obscure subjects as perigyny and epigyny.

When we consider the flower as a whole, rather than its individual parts, we find that the general construction is influenced in the main by a third factor—the shortening of the internodes in comparison with those of the average vegetative shoot, and the resulting intimate relation which often subsists between this telescoped floral shoot and its parent axis and axillant bract. These influences result in a close packing of the members, and favour cohesion and adhesion, as well as distortion and suppression of parts, and various forms of deviation from full symmetry. In studying the results of such factors, the earlier workers after Goethe, such as A. P. de Candolle and his followers, relied entirely upon the external form-relations, and those features of the nervation which were seizable by the naked eye; the brilliant results which they obtained showed how much the comprehension of the flower could be advanced without reference to internal

structure, or to ontogeny as now understood. However, before long it became evident that these primitive methods had already yielded the best that could be expected from them when unsupplemented. This was perceived at a very early stage by de Candolle's contemporary, Robert Brown, who turned to the microscope for further aid, and proceeded to lay the foundations of floral anatomy—somewhat casually, as was his wont, in the midst of papers largely occupied with other matters (8, 9, 10). In England little attention seems to have been paid to floral anatomy between the days of Robert Brown and George Henslow (17, 18). Henslow was influenced in great measure by the writings of van Tieghem, whose memoir on the gynaeceum (25) remains the most comprehensive and fully illustrated study of floral anatomy extant[1]. This work was a thesis on a subject set, in 1866, by the *Académie des Sciences*. The problem proposed for research was the anatomical structure of the pistil and fruit, which was suggested because "il reste sur plusieurs points des doutes, que l'examen anatomique de ces organes, à diverses époques de leur développement, pourrait probablement resoudre." I cite this sentence because of the modest nature of the hopes which it expresses. Henslow approached floral anatomy in the same reasonable spirit. He merely says, "I am convinced that it will enable us to advance some way nearer to the solution of several morphological problems, if it do not actually solve the difficulties which have beset the investigations of floral structures" (18). It is indeed unfortunate that this moderate attitude has not been maintained, and that later workers have become so much hypnotised by anatomy that they have attempted to isolate it, and to treat the vascular bundles as if they were entities independent of the organism of which they form a part. This position may, of course, be defended on the ground that anatomy—in common with other scientific disciplines—is, in its very nature, an abstraction from reality. This is perfectly true, and the fact of this necessary abstraction does no harm so long as we remain alive to it; but it is apt to be ignored, and then anatomy—isolated and used as a mere source of phylogenetic indications—is set hopelessly adrift. Its disorientated state is the inevitable outcome of treating the conclusions from analysis as if they were the conclusions from a holistic study, and of forgetting that end-results can never attain to a higher order of validity than the premises on which they are based.

It cannot, indeed, be too strongly emphasised that external shape

[1] For an historical survey of the nineteenth century writings on floral anatomy, following van Tieghem, see Grélot (15).

and anatomical structure are not discrete entities, but are merely two aspects of "form"—using this term in its broad morphological sense. For the external form, and the ground-plan of the vascular system, are both end-products of differential growth and development in the same mass of tissue. In ontogeny, the *process* of this differential development is studied; ontogeny is therefore not a separate subject, but is merely the developmental aspect of form and anatomy. It follows that the study of the external form, the vascular system, and the ontogeny, should not be pursued as three separate branches, but should be treated as one indissoluble whole. Their separation is an example of that particular type of analysis without subsequent reintegration to which the British mind seems particularly prone; in this country a reaction in favour of the holistic view of the plant organism is long overdue. In modern German morphology, on the other hand, one cannot but feel that this reaction has gone to somewhat startling lengths. Wilhelm Troll's book on the flower(27) is an example of the excessive swing of the pendulum away from that analytic mode of thought which reached its zenith in the nineteenth century. Troll claims that his view of "form" (Gestalt) is that upheld by Goethe, but, after a careful study of *Die Metamorphose der Pflanzen*(14), I have come to the conclusion that Goethe's views were more strictly scientific, and less nebulous and mystical, than those which Troll adopts[1]. Troll considers that form cannot be understood as a function of the material substratum, or of the processes going on within it; he holds that it is "eine Totalität oder Ganzheit" which lies above the causality level, and hence cannot be comprehended or explained on causal (physiological) grounds. According to this view, form is a unity which is, in its very nature, unanalysable. Troll considers that morphology—in contrast to the analytical science of physiology, which proceeds from the parts to the whole—begins with the totality, and passes to the individual forms. He puts aside "explanation," in the sense in which that word is used in the exact sciences, and treats it as having no place in morphology. Morphology, in his view, starts from the intuitively-seized totality of the organism, in the same sense as that in which physics may be said to start from the intellectually-apprehended idea of the electron.

Though the approach to mysticism in Troll's attitude is alien to the mental atmosphere in which natural science is pursued in this

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[1] Goethe's attitude is, I think, represented more fairly in an earlier study by Hansen (16).

country, there is undoubtedly an element of real value in the stress which he lays on the study of form as an independent discipline. A general discussion of his position would lie outside the main subject of this article, so I will only say that, if one adopts his synthetic and holistic attitude so completely as to exclude analysis, scientific work, in the strict sense, comes to a full stop. The position taken up by Dr E. S. Russell, in his recent work on development and heredity (19), appears to be a much more reasonable one. Though his sympathies are all with the "organismal" standpoint, which is essentially synthetic, he is careful not to rule out analysis, provided it is invariably followed by reintegration. His attitude offers—in theory, if not entirely in practice—a sane compromise between the exclusively analytical and the exclusively synthetic positions. It is greatly to be wished that someone would produce a treatment on broad lines of the *botanical* conception of the organism, to balance and supplement Russell's brilliant exposition, which is basically zoological.

Whatever may be thought of the modern trend towards a holistic biology, it has at least the great merit of encouraging the study of morphology for its own sake and as an end in itself, rather than for its real or fancied utility in tracing phyletic lines. If this independence is consistently maintained, it may lead to a renascence of morphology after its long period of decadence, which dates back to the publication of *The Origin of Species*. One of the regrettable results of the revolution in biology initiated by Darwin was a loss of interest in morphology, except in so far as it could be coerced into serving as an adjunct to phylogenetic speculation. Those problems of organic symmetry which had at least been steadily envisaged— if not solved—in the pre-evolution days of A. P. de Candolle and his pupils, were inevitably shelved when thought was directed into teleological channels; for characters involving number, pattern, and symmetry-relations have seldom an obvious survival value. Moreover, Darwin's work unfortunately favoured the notion that to say that a character is "due to inheritance" suffices as an *explanation* of that character. For instance, in his study of the Orchid flower, writing of "the fundamental framework of the plant, such as the remnants of the fifteen primary organs arranged alternately in the five whorls," he says that nearly all those who believe in the modification of organic beings will admit that the presence of these remnants "is due to inheritance from a remote parent-form" (12). Darwin thus relegates the essential problem—the mystery of "the fundamental framework"—to remote regions with which he feels no need to con-

cern himself. In this country almost all the biologists of the nineteenth century adapted their thought to the limitations imposed by Darwin. One of the exceptions was William Bateson, who, in his *Materials for the Study of Variation*(7)—his first and most original theoretical work—showed himself keenly alive to the problems of symmetry; but in the later phases of his research his thoughts were deflected from *form* to the *inheritance of form*, while symmetry, considered in itself, ceased to engage his attention.

Even at the present day, though Darwin's influence has waned, the problem of symmetry is still in great measure ignored. For the key note of botanical thought is now set by the physiologist, who is naturally little drawn to problems of organic symmetry, since he himself deals with a facet of reality from which form is necessarily excluded. Among the few English books of this century which deal seriously with the question of symmetry, Prof. D'Arcy Thompson's *Growth and Form*(24) must be mentioned; but, though full of suggestion for the botanist, it is essentially a zoological work, in which the plant makes rare appearances as a slightly embarrassed alien. The time is still to come when the attention of botanists will be deliberately focussed upon the problems of symmetry. When this happens, there is little doubt that questions of form and anatomy will assume a fresh orientation. The lines which this re-orientation will follow are hidden from us, but I think it cannot but involve a belated realisation of the fact that external form is not the cause of the vascular ground-plan, nor is the vascular scheme responsible for the external form, but that both are collateral manifestations of the basic symmetry of the plant. Botanists will then no longer be tempted to abuse floral anatomy by treating it as a reliquary to be rifled for "ancestral traits," but the pattern of the vascular system in the floral shoot, and the relations of the parts of the flower, will both be recognised as the expressions of a certain underlying growth-rhythm, inherent in the constitution of the species. When that point is reached, the morphological interpretation of the flower will no longer be pursued piecemeal and in detachment, but will fall into place as part of the general problem of the symmetry of living things.

SUMMARY.

It is held that the differences between the flower and the vegetative shoot are conditioned in the main by three factors:

(1) The divergence of the floral members from the foliage leaf

type, in correlation with the difference between sporogenous and vegetative activity. In the carpel this involves the shifting of activity from the midrib to the margins.

(2) The peculiar relation of the ultimate leaf members (carpels) to the apex of a shoot of limited growth (the floral axis).

(3) The telescoping of the floral axis, and its intimate association with the parent axis and bract, which leads to close packing of the appendages, and favours cohesion, adhesion, and various forms of distortion, suppression, and departures from radial symmetry.

With this theory of the flower as a basis, the phylogenetic claims of floral anatomy are considered. It is held that the *general* scheme of the vascular system may have some value as indicating the broader trends of race history; but it is shown by a study of a series of rudimentary leaves, sepals and stamens, that there is no positive evidence for the alleged "conservatism" of the vascular bundles—or, in other words, for their survival when the organ which they supplied has ceased to exist. This conclusion, which confirms the result reached long ago by Grélot (15), involves the rejection of various phylogenetic speculations.

As offering an extreme contrast to that partial and narrow outlook upon anatomy from which the vascular bundles are seen as isolated entities, the conception of "form" encountered in modern German morphology is briefly considered.

Finally a plea is entered for a treatment of flower structure and anatomy, not in detachment from morphology as a whole, but as part of the great plexus of problems in organic symmetry, which are at present so inadequately realised that we still await, not only their solution, but even their full formulation.

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REVIEWS

J. BRAUN-BLANQUET and E. RÜBEL. *Flora von Graubünden*. Erste Lieferung. (Heft 7 der *Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich*.) Pp. 382 with one map. Bern und Berlin: Hans Huber. 1932. Fr. 22.50.

The Graubünden is the south-eastern quarter of Switzerland and includes the valleys of the upper Rhein, the Inn and their tributaries, as well as those of a few rivers flowing southwards into the Italian lakes. For the purposes of the flora this region is divided into three major sectors by each of two systems. The first system is used for plants of elevations less than the subalpine stage, and it comprises (*a*) a north region with beech dominant in the valleys—here the precipitation and relative humidity and incidence of mist are high (precipitation 900–1500 mm. per annum), (*b*) a central region much larger than the others, with pine dominant in the valleys—here the precipitation is lower (600–1000 mm. per annum), there is lower humidity, less mist and increased insolation, and (*c*) a southern region with oak or mixed deciduous woods—here the yearly mean temperature and insolation are higher, but so also is the precipitation (1400–1600 mm. per annum). The system of division used for the sub-alpine and alpine plants differs somewhat from this and follows more closely the geological groupings of the various mountain systems, though similar north, central, and southern sections are made.

The present volume of the flora deals with the Pteridophyta, Gymnosperms and the Monocotyledons, the arrangement and nomenclature being that of Schinz and Keller. A large number of eminent Swiss botanists have assisted in the compilation of the data, and reference is made to many herbaria. As one would expect from the deep ecological interests of the authors, the habitat conditions of the species are given with unusual care and precision. It will probably be of value to give by means of an example the form typical of the average short entry.

"*Juncus subnodulosus* Schrank (*J. obtusiflorus* Ehrh.).

"Selten und nur in tieferen Lagen, aber wo vorhanden meist herdenbildend, an wasserzügigen Stellen, Grundwasseraustritten, im Molinietum und Schoenetum nigricantis auf kalkreichen, basischen Böden. Steigt bis 1100 m. bei Fardün im Schams (B.-B. in Sched. Fl. raet. exs. Nr. 1032)."

Then follows a list of occurrences in the three regions, with citations of altitude and collectors.

It will be seen that the volume will not only be extremely valuable to the Swiss botanists but will be of great value to phytogeographers and ecologists interested in any of the general problems of the distribution and history of the flora of Europe.   H. G.

RüBEL, E. *Ergebnisse der internationalen pflanzengeographischen Exkursion durch Rumänien,* 1931. (Heft 10 der *Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich.*) Bern: Hans Huber. 192 pp. Fr. 8.

The sixth International Phytogeographical Excursion was held in Rumania in the summer of 1931 under the leadership of Prof. Borza, of the University of Cluj. The present volume gives the constitution and itinerary of the party and a series of original papers by various participants, on topics connected with the ecological and phytogeographic problems of the excursion. This review can be most useful by indicating briefly the content of these various papers.

Prof. Borza gives a critical revision of *Cerastium transsilvaticum,* a species belonging to the *C. Alpina* group and endemic to the south Carpathians, where it is found at elevations between 1500 and 2500 m. He considers it to be derived from the Tertiary high mountain plant, *C. Alpina*: from the three varieties now existing he argues an origin before the glacial period and perglacial survival, whilst its endemism he attributes to its slight capacity for horizontal spread.

Dr Krajina gives a careful revision of the systematics and distributional data of some of the species of *Festuca* from the montane, alpine and sub-alpine stages of the Rumanian Carpathians. Especially interesting is the discovery of the occurrence of *F. eskia* Ramond., in these mountains. It thus has a Pyrenean-Transylvanian distribution area, with a marked Alpine hiatus, as also have *Gentiana pyrenaica* and *Carex pyrenaica.* The account includes diagnoses of eleven species of *Festuca.*

A further systematic paper is contributed by Dr Nyárády who describes species of *Poa* collected on the I.P.E. excursions in the southern Siebenbürgen Carpathians.

A more general view of the vegetational relationships of the country is given by the articles of Profs. Regel and Domin. Prof. Regel gives a general comparison between Rumania and Lithuania. There are of course many large differences such as those due to the presence in the Rumanian flora of Tertiary relics, and the presence in Rumania of Pontic, Central Asiatic and Mediterranean elements as contrasted with the Atlantic, sub-Atlantic and Boreal elements in Lithuania. At the same time the resemblances are surprisingly

great, and this is particularly marked with regard to the aquatic communities: this fact, which is in accord with the general experience of phytogeographers is even more strikingly brought out by comparing the lists of aquatic plants from the delta of the Danube with similar lists from the English Fenlands. There, perhaps on account of the milder English climate, the degree of correspondence is much greater. The vegetation of the oak and spruce forests show resemblances also, but comparison with the Rumanian beech forest is not possible since Lithuania lies beyond the north-westerly limit of the beech distribution area in Europe.

Prof. Domin describes the major plant communities met with successively as the I.P.E. excursion ascended from Sinaia (850 m.) to the summit of Mt Omul (2508 m.) in the centre of the Bucegi massif, and then descended on another flank of the mountain. The general disposition of the altitudinal zones in the Rumanian Carpathians is as follows:

<div style="margin-left:6em">

Alpine
Sub-alpine
2000 m. ....
Coniferous forest (*Picea abies*)
1400 m. ....
Beech forest (*Fagus sylvatica*)
850 m. ....
Oak forest (*Quercus robur, sessiliflora, cerris* and hybrids)
300 m. ....
Avant-steppe (sparse and dwarf tree growth).

</div>

Interesting variations from this sequence are given by Prof. Domin in his record of the zones on the approach to the Omul, in (*a*) the mixed beech-fir zone above Sinaia (Fagetum-abietosum) at about 1050 m., and (*b*) the record of *Larix carpatica* at about 1350 to 1850 m. on steep slopes where spruce competition appears to be less severe.

Lastly we should mention a short paper by Dr Bujorean giving the results of detailed recording of the microclimate of the small hillocks (ţicle) in the Siebenbürgen region.

The whole forms a valuable and interesting book, but its utility and appeal would probably have been much widened had it been possible to print a concise general account of the major ecological and phytogeographic features seen by the Excursion, or of the country as a whole. It would perhaps have been unkind to have added this task to the herculean efforts made by Prof. Borza in organising the whole excursion, but those who participated in it will recognise how satisfactorily he would have achieved a preliminary article of this kind. The broad general relationships of the vegetational regions of the country to the major controlling factors of climate, soil, and biotic influences, are of the greatest interest and this seems precisely where they could be appropriately described.　　　　　　　　　　　　　　　　H. G.

THE BEHAVIOUR OF DYES IN THE TRANS-
PIRATION STREAM OF SYCAMORES
(*ACER PSEUDOPLATANUS* L.)

BY H. BAKER AND W. O. JAMES

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(With 7 figures in the text)

INTRODUCTION

NUMEROUS investigators have used dyes in their efforts to trace the movements of water inside plants. It must be admitted, however, that the methods used have not always been well designed to fulfil their purpose, and the conclusions arrived at have sometimes been so inherently improbable that further observations seem highly desirable. By the courtesy of St John's College we were able to make numerous experiments on sycamores growing in Bagley Wood above Oxford. A plantation was cut in 1925 and by the autumn of 1930 the stools had produced numerous shoots, the oldest being 4 m. or more high, 5–6 cm. in diameter towards the base, and showing five annual rings. Our experiments were carried out mainly on four- and five-year-old stems and extended throughout the year, including both winter and summer seasons. The results obtained in the summer, with expanded leaves and high rates of transpiration, differed so completely from those of the winter that a separate treatment is called for. This paper is concerned in the main with the first set of results, and an account of the winter movements is given in a second.

Various methods of introducing the dye have been practised by different workers, an inevitable technical fault being that the manipulation always disturbs the normal conducting system. The basic difficulty lies in the fact that the conduction probably occurs in an almost closed structure with the water under tension, and dyes, to which living tissues are impermeable, cannot, therefore, be introduced

without opening this unit in some way or another so that the tension is released. MacDougal, Overton, and Smith (1929) claim to have overcome the difficulty by introducing dye solutions into the trunks of large trees by bores lined with hollow brass tubes attached to tubular glass reservoirs. The method is an old one and was perhaps invented by Hales in the eighteenth century (see also Schwendener, 1888), but similar claims have not been made on its behalf by other workers, and it will become apparent that they cannot be entertained even for individual tracheae not actually cut by the boring, especially as MacDougal, Overton, and Smith even used a hand pump to force the water well into the wood. The authors themselves comment on the similarity of the "pattern" obtained when their "unopened" systems are compared with others they admit to be opened.

It is probably best to admit at the outset that the ideal of getting something into an enclosure without unclosing it is impossible of attainment in practice. We do not claim for any of the methods described below that they get dye into the conducting system without any disturbance of it, but prefer to consider the different artificial systems set up, and to endeavour to deduce from them the behaviour before disturbance.

Methods

All experiments were carried out in the natural habitat. When the time for the movement of the dye had elapsed the shoot was sawn off at the base, expanded leaves trimmed away, and the material hurried by car to the laboratory in Oxford. Examination was carried out as soon as possible, a reasonable precaution against any subsequent slow creeping of the dye. To discover the distribution of the colour at any given level the shoot was sawn across, and a clean face trimmed with a razor. A slow and slight spreading of the dye occurred after cutting, but error from this source could be avoided by ordinary alacrity. Comparison of the two cut faces afforded a useful safeguard, and the fact that cuts near a bore always gave a sharp projection of the bore's diameter showed that the observations were satisfactory. Further confirmation was often obtainable by splitting short lengths longitudinally in any required position.

Dyes were applied to the stems by methods falling under four headings.

   (a) Stepping cut branches into pots of solution;
   (b) boring under water and replacing the water with dye solution;
   (c) boring under water and stuffing the bores with dye crystals;
   (d) application of dye solutions to the surface of exposed wood.

Whenever a bore was to be made a plasticine cup was first fixed to the stem, and filled with distilled water or dye solution. Boring was then carried out on a downward slant with a 6 mm. steel drill. If the dye was to be supplied in solution the plasticine cup was left in position, or alternatively a threaded brass tube was screwed in, and the dye supplied through this without additional pressure. If solid dye was to be given boring was carried out under water as before, and after a short time the cup was removed. If this was done without undue jerking the bore remained full of water, and considerable amounts of solid dye could be introduced on a fine knife-point, while at the same time by judicious irrigation the bore could be kept filled to the brim with water. The hole was then hermetically sealed by windings of insulating tape to prevent any entry of external air as water was taken up from the cavity. This method represented the nearest approach to a natural system, since after a time normal tensions might be re-established, there being no continuous artificial supply of the liquid. If the experiment lasted for any considerable length of time, however, the escape of internal gases into the cavity again disturbed the normal working.

Two dyes were employed, both at approximately 1 per cent. strength when supplied in solution. Acid fuchsin was selected as an electronegative dye, previously used by other investigators, and methylene blue as an electropositive dye having the additional advantage of causing the minimum of injury to living cells.

The path of movement

In points of detail different experiments yielded different results according to the method used and the external conditions prevailing, but all experiments, by whatever method and whatever the time of the year they were performed, agreed upon two points. There was never any appreciable travel of the dyes in bark or pith, and the dyes always passed both upwards and *downwards* from any point of application other than a cut end. In one experiment a layer of living cells less than 0·5 mm. thick prevented any access of dye to the wood. This high resistance of the bark has, moreover, been the experience of numerous other investigators, and so need not be discussed in further detail. Downward movement of dye solutions in manipulated transpiration streams has also been described by Dixon (1924) using eosin in potato and elder; Birch-Hirschfeld (1920) applying eosin to a variety of species; and Arndt (1929) with eosin in coffee trees. Its existence, therefore, need cause no surprise, though it seems to have been

overlooked in some of the attemps made to elucidate the course of the transpiration stream through woody stems by these methods.

It is reasonable, having regard to the complex structure of wood, to suppose that the transpiration stream does not pass through all its parts indifferently. From the detailed examinations we carried out reconstructions of the path of the dye could be made (see Figs. 1, 2) and these reveal some important features. In the experiment of Fig. 1 acid fuchsin was carried into the innermost annual rings of a four-year-old stem. A leakage of dye obviously occurred between the sides of the bore and the brass insert, and although the innermost rings received the most copious supply, the third annual ring appears to have transported about as much as either the first or second. Perhaps the most interesting feature is, however, the change over of the dye between 10 and 15 cm. below the bore and between 25 and 30 cm. above it. It is clear that isolated cuts at 15 cm. below or 30 cm. above would have convinced the experimenter that conduction occurred only through the porous early ("spring") wood of each annual ring; while cuts at 10 cm. below and 25 cm. above would have convinced him that it lay through the closer late ("summer") formation. Similar examples from other experiments are not lacking, and it seems that the most facile path of conduction shifts easily from one radial position to another within the annual ring. The radial shift even seems able to cross the division between one year's growth and the next without serious difficulty. There is a strong suggestion that this occurred from the first to the second ring both above and below the bore and, perhaps, from the third to the fourth ring between 25 and 30 cm. above. Radial shift from the first to the second ring and from the third to the fourth ring is also shown in Fig. 2. Tangential shift was also observable and showed itself first as a lateral spreading of the dye in cross-section, and finally in the position of the most distant coloration detectable, since this was rarely vertically above the bore.

Both the above experiments were carried out by supplying dye solution to a bore, but a further experiment in which a four-year-old branch was cut off and stepped into a bottle of 1 per cent. acid fuchsin showed no essential differences in the upward movement. It is significant that although the dye was presented uniformly to the cut surface in this experiment uptake was irregular, and appeared to be pre-eminently through the early wood of each annual ring. A cut 23 cm. up the branch showed a reversal of this distribution: the last-formed ring contained no dye at all, but in each of the others it lay
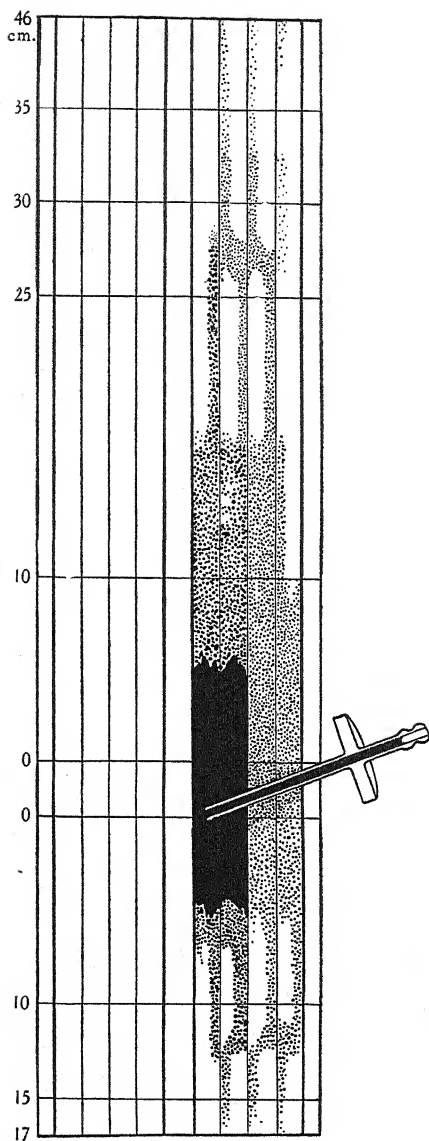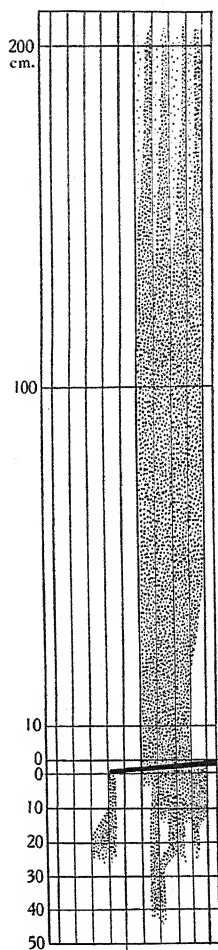
Fig. 1.   Fig. 2.

Fig. 1. Diagrammatic reconstruction of the path of acid fuchsin solution through
a four-year-old stem. The depth of shading represents approximately the
depth of colouring observed. Pith and bark are separated from the wood
by heavy lines, annual rings by light lines. Horizontal scale five times the
vertical scale.

Fig. 2. Diagrammatic reconstruction of the path of acid fuchsin through a
four-year-old stem. Details as in Fig. 1, but horizontal scale 12·5 times
vertical scale.

principally in the late wood, and only a small sector of each circle was coloured in contrast with the complete staining at the base (Fig. 3).

A series of experiments was carried out by method (d) as follows. Four plasticine cups were fixed to the stem at intervals of a foot apart. The cups were filled with water and a strip of bark removed in each. To avoid opening vessels at the surface of the exposed wood the strips were taken by making two vertical cuts about 1 cm. apart and 4 cm. long, and then lifting the bark with the horn handle of a budding knife. After this the loosened strip could be cut away without any danger of the knife coming in contact with the wood and any chance of incision was limited to the two vertical cuts. The plasticine cups were numbered from below upwards and the water was replaced by in-filtration of acid fuchsin. At each cup the dye slowly penetrated into the wood all over the exposed surface and not merely at the sides where damage may have occurred. After 24 hours upward and downward travel had been extensive, the upward stream from the bottom cup overlapping the downward stream from the one above, while the upward movement from the third had reached the leafy crown of the shoot. There was also consider-able radial penetration into the wood, reaching as much as 6 mm. in some places and involving passage from
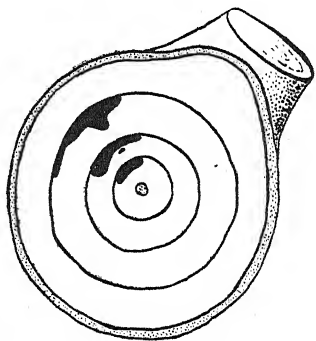
Fig. 3. Cross-section showing distribution of dye (full black). Wood white, bark shaded.

the outermost (fourth) annual ring into the middle of the adjacent third, the average width of an annual ring being about 4 mm. Radial penetration was also shown by the fact that the course of the dye could be traced to a greater distance from the point of application by sectioning than by stripping and observing the surface of the wood. The tangential spread of the dye at its greatest was about twice that of the exposed surface of wood. In similar experiments, where the surface of the wood was deliberately or accidentally scratched, heavy streaks of dye were always formed above and below the scratch, in-dicating a relatively rapid absorption at these places. Failure to remove all the bark from the face of the wood meant, on the other hand, that no penetration would occur however thin the residual layer.

The above observations were carried out by naked eye or with hand lenses giving a magnification up to eight times, and to amplify them thick sections were taken at selected places. The ordinary methods of cutting under water are obviously useless for the present purpose, and to prevent spreading of the dye the wood was first cut into blocks under liquid paraffin, and further sectioning carried out with a razor flooded with the same medium. No attempt was made to get thin sections, and in longitudinal cuts the smaller elements,
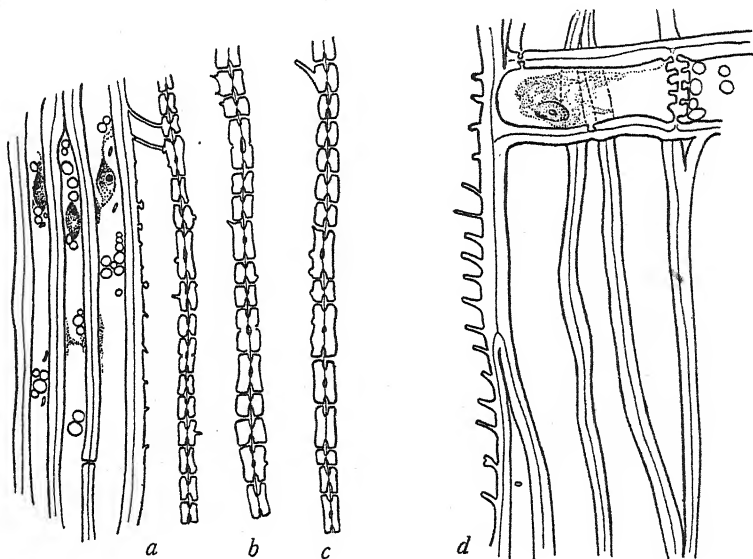
Fig. 4. Radial longitudinal section of a group of vessels, *a* ⟶ *d*, "substitute fibres" with starch grains on left, true fibres on right. Bordered pits are shown in the tangential walls between vessels. Drawn with camera lucida from fresh material treated with iodine and mounted in glycerine. 1/12 in. obj., × about 1100.

fibres, etc., were left undamaged: the larger vessels were invariably opened. As a result dye was not usually found free in the larger tracheae though its original presence could be proved in an interesting manner. The vessels of sycamore are solitary or in radial groups of about four or five. On the radial walls separating vessels from starch-containing fibres only a few scattered and slit-shaped simple pits are found, and the walls appear smooth in surface view save for some narrow oblique bands of thickening (Fig. 4). On the tangential walls separating one vessel from another numerous bordered pits are

formed. These have oval openings, a thin membrane, and are so closely arranged that they form a hexagonal panelling in surface view: there are no oblique bands (Fig. 5). When longitudinal sections were
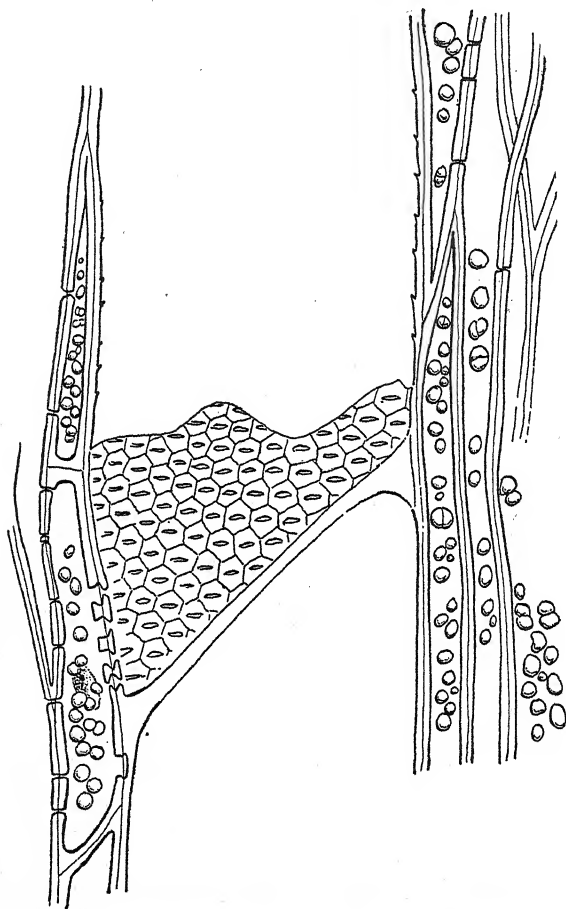
Fig. 5. Tangential longitudinal section of a vessel and adjoining parenchyma (left), and substitute fibres (right). Drawn as Fig. 4.

cut under paraffin the only dye invariably visible was found in these bordered pits. In tangential sections they showed as a delightfully coloured tesselation with bright ovals of colour at the mouths of the pits surrounded by paler hexagons where the enclosed dye in the

cavity showed through the pit borders. In radial sections the dye could also be seen occupying the whole pit and the menisci at the pit openings could be observed: even in pits that appeared to be opened by the cutting the dye was retained. The invariable presence of the dye in the bordered pits and its absence from all surrounding positions can only be explained on the supposition that movement of the dye occurs via the cavities of the vessels, and that passage from one vessel to another is very easy through the numerous bordered pits. In sharp contrast with the brilliant colour of the pits the material of the walls remained completely unstained, and it was only after prolonged exposure that it took up the dye. These observations could be made both with acid fuchsin and methylene blue.

From the foregoing it seems certain that rapid movements of the solutions under the influence of transpiration are limited to the lumina of the vessels, and such a conclusion is in agreement with general opinion at the present time. The direction of the passage will, therefore, be determined by the course of the vessels, which occupy only a very small proportion of the total cross-section of the wood (especially in sycamore: see Atkins, 1916; also Strasburger, 1891, etc.). The groups of vessels do not ascend the stem in completely isolated vertical rows, however, but sway from side to side and link up at various points

Fig. 6. Tangential longitudinal section of young wood showing anastomoses of vessels. Cells with living contents conventionally shaded. Note the medullary ray passing between the vessels just below their junction. Drawn with Watson projection apparatus from fresh material treated with iodine and mounted in glycerine. 1/6 in. obj., × about 500.

with adjacent series, so forming a complex network throughout the

xylem. We thus have to do, not with a single channel and direction of transport, but with a maze of channels reminiscent of those of a complex delta through all of which the water moves *more or less* in the same direction.

One of the most interesting cases of radial displacement to come under our notice was that of a vessel series injected with acid fuchsin
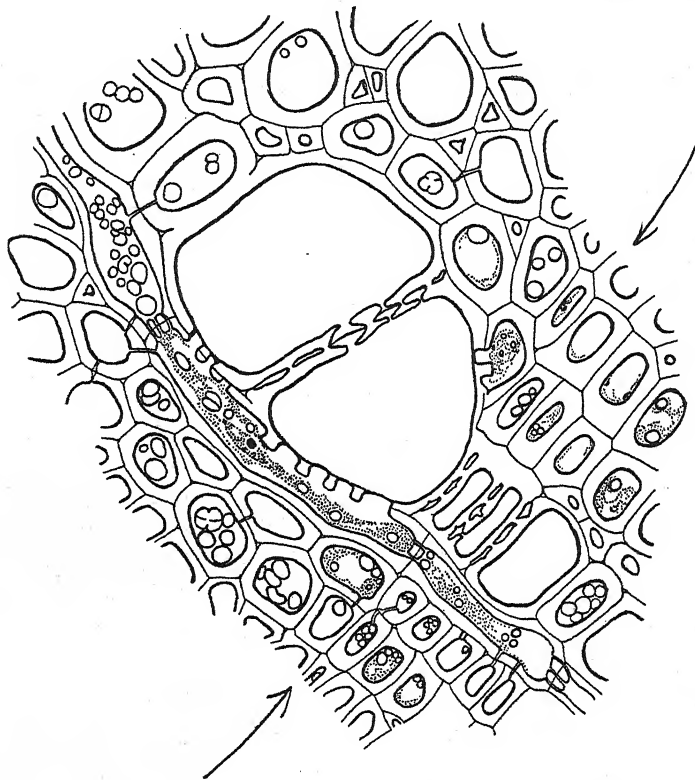
Fig. 7. Transverse section showing border between two annual rings, and a radial series of vessels continuous across the junction. Note bordered pits on all tangential walls. Drawn as Fig. 4.

by scratching the surface of the wood. It approached the cambium only at one point and owing to the coloration of its bordered pits could easily be traced up and down in longitudinal section. Within the limits of an ordinary freehand cut (about 1 cm. long) it had passed well into the interior of the first annual ring, and at a short distance below the point of injection fourteen fibres lay between it and the cambium, more than are usually found separating one vessel

series from the next. Such displacement is, moreover, by no means rare nor limited to any particular region of the wood. Casual inspection of any reasonably extensive longitudinal section will show vessels in contact at one point and separated by bands of fibres at no great distance (Fig. 6), and similar observations have been recorded by Zimmermann (1923) for *Salix Babylonica*, using a very ingenious method of serial sectioning. It is of particular interest to note that connections between vessels may occur across the division of one year's growth from the next (Strasburger, 1891). We were at particular pains to examine these junctions, as they might clearly be expected to form the passage by which the water passes from one annual ring to another as shown in the macroscopic observations. An example is illustrated in Fig. 7, the most significant fact in the present connection being the development of normal bordered pits on all the tangential walls including that separating the small vessel of the late wood from the large vessel formed in the following year. There appears to be no more reason why water should not pass between these two vessels than between any other pair, and the existence of anything like complete impassability between annual rings is, therefore, extremely unlikely.

THE RATE OF MOVEMENT

By measuring the extreme distance to which the dye had penetrated and keeping a record of the duration of the experiment it was

TABLE I

Date			Dye	Rate of penetration in cm. per hour		Method
				Upward	Downward	
1930 Sept.	8		Acid fuchsin	32	52	Shallow bore; 1% dye solution
	11		,,	80	40	Bore with brass tube; 1% dye solution
	11		,,	100	24 ⎫	Wood exposed
	11		,,	81	16·5 ⎭	and scratched
	18		,,	1·25	0·25 ⎫	Wood exposed
	18		,,	1·16	0·25 ⎬	but not
	18		,,	2·10	0·23 ⎭	scratched
	18		,,	7·5	1·66	Bore; 1% dye solution
1931 June	23		,,	600	35	Bore; 1% dye solution
Aug.	20		Methylene blue	1·29	0·5 ⎫	
	20		,,	4·13	0·5 ⎬	Bore; solid dye
	20		Acid fuchsin	6·00	0·66 ⎪	
	20		,,	5·5	0·76 ⎭	
1930 Sept.	15		,,	50	50	Defoliated shoot; bore; 1% dye solution

possible to calculate approximately the rate of travel of the dyes in both directions. All the relevant results are given in Table I.

It is clear that the rate is dependent on occasion, the direction of movement, and the method of application of the dye. The very rapid rates recorded on June 23rd, for example, were associated with ideal conditions for rapid transpiration, and contrast markedly with the rates obtained by the same method in September. An important distinction also lies between the rates observed with the open bores and simple application of the dye to the wood surface (experiments of September 18th), and it can only be supposed that the slow penetration of the dyes through the unbroken face is due to the resistance set up to their passage on first entering the vessels. The rapid rates of movement found with open bores and cut stems are probably quite different from the normal movements, which in this respect may be supposed to resemble much more closely the slower rates found with surface application and, perhaps also, with insertion of the dyes in solid form.

More important than the actual rate is the ratio of the rates upward and downward. These are summarised in Table II.

Table II

Season	Number of experiments	Mean ratio $\dfrac{\text{Upward rate}}{\text{Downward rate}}$	Remarks
June	1	17·14	
August	4	6·79	Leaves spread
September	8	4·38	
September	1	1·00	Artificially defoliated
Winter months	16	1·30	Leaves off

(Details of the winter experiments will be given in a later paper.)

Taking into account the method of applying the dye, the winter months may be compared directly with August and the defoliated shoot with the June result: the September experiments include a variety of methods (see Table I). These comparisons show at once that, when the leaves are expanded and transpiration rapid, the ratio rises considerably above unity (upward movement the more rapid), but when transpiration is stopped by artificial or natural removal of the leaves the two rates become equal. This result agrees with an observation of Arndt (1929) on coffee trees. He found that injections of dye in the daytime, transpiration being rapid, gave rise to more rapid movement upward than downward, but that at night when

transpiration was reduced the two rates were approximately equal or the downward movement might even become the greater.

These observations may not appear at first sight to be in accord with the idea that the movements of the stream depend on liquid tensions striving to operate in all directions equally, but that the tension on the water is in fact equal in the upward and downward directions can be shown simply. If the surface of the wood is stripped, a pool of dye applied, and the wood then stabbed with a scalpel flooded with dye, the colour may be seen to enter rapidly both up and down the severed vessels. The mean ratio of upward and downward movement in thirty-four experiments was 1·03, and it made no appreciable difference to the ratio whether the leaves were wilted or not, or whether the twig was on or off the tree.

There is thus no case for invoking unequal tensions in the two directions, the inequality of the penetrations over long periods being due to the conformation of the wood. The general tendency while transpiration goes on is for water to move upward. This tendency is destroyed below the bore in the vessels actually ruptured, and the existing tensions then tend to draw water down them. This downward stream is, however, comparatively local, and dye is continually lost from it via anastomoses with vessels whose water is still moving upwards, and these losses inevitably reduce the net downward movement. When transpiration is brought to a virtual standstill by loss of the leaves the tensions are not instantaneously abolished, but persist for a considerable time (see also second paper). Under these conditions there is, however, no upwardly directed stream of water and the dye passes equally in both directions and the ratio becomes one.

Conclusion

The picture we arrive at represents the transpiration stream as passing up the stem in a multitude of fine branching and rejoining channels formed by single or grouped vessels threading their way through a non-conducting matrix. The matrix is built up of living parenchyma and non-living fibres, but for the most part the fibres can only be approached from the vessels by passage through one or more living cells. There may be, and probably are, complex interchanges of water and nutrient substances between the water streams and the cells bordering them, but the quantities of water involved in these exchanges are quite negligible compared with the masses being moved by transpiration. There seems no reason to doubt the well-accepted hypothesis that the water of these streams is under con-

siderable tension, and in a complex system such as the wood chance alterations of resistance will necessarily change the quantity of water passing through any particular channel. Such changes have often been induced artificially as in the experiments reported above and in other investigations (Dixon, 1924; Arndt, 1929; Zimmermann, 1923): these have led to the predictable local changes of rate and direction of flow. We find nothing, however, either in our own or other workers' experiments to suggest that large deviations from the general upward movement occur in any natural and unmanipulated system. We cannot, therefore, envisage the xylem and the transpiration stream as normal vehicles of downward translocation as has been suggested.

It has at various times been shown (e.g. Caldwell, 1925 and 1930; see Zimmermann, 1923, for older experiments) that dyes applied to a particular root, petiole or side branch are drawn only into certain leaves having some simple anatomical relation. This has been taken as evidence that the channels in the xylem are separated from one another laterally more or less completely. This view finds its most rigid expression in a recent publication by MacDougal, Overton and Smith (1929), whose results are given in diagrams reduced to simple geometric formations. The accompanying text implies that these diagrams are to be taken literally: thus speaking of *Salix lasiolepis* they say "This restriction of the dye to the late summer wood produces a pattern of concentric coloured and uncoloured rings in the transversely cut face of the trunk." *Alnus oregona* showed them complete rings limited to the spring wood, and the two cases are illustrated by an appropriate number of firmly drawn circles, and equally diagrammatic longitudinal reconstructions. As shown above we were unable to find anything approaching this rigidity of separation in sycamore wood, and the methods employed by MacDougal, Overton and Smith leave one in serious doubt of the validity of their picture for the other species. It has already been pointed out (p. 248) how easily one might be misled into over-simplification by cuts spaced too far apart, and MacDougal and his assistants give no information of how close or how numerous were the sections their reconstructions were based on. Furthermore, incredible as it may seem, they left their wood to dry for long periods, sometimes more than a year, before examination, altogether disregarding the danger that the dye might move in the meantime. In addition they rest a faith, which we find quite impossible, upon the method of injection by bores, and finally they looked for but failed to find any anatomical basis for their curious suggestions.

The more or less vertical ascent in normal circumstances thus depends on a smaller degree of resistance in that direction rather than on a complete blockage in all others. Should a vessel be blocked by bubble formation or other mishap the stream leading to it will be diverted to other channels by way of the radial or tangential anastomoses. The fact that there are islands of non-conducting tissues does not introduce any fundamental difference in this respect from the behaviour in coniferous wood as originally described by Dixon (1914).

Summary

1. The passage of acid fuchsin and methylene-blue solutions through the wood of young sycamore stems was observed under a variety of circumstances.

2. Except where application was made at the cut end of a stem movement of the dye invariably took place both upward and downward.

3. Movement never occurred in bark or pith.

4. Radial and tangential shift occurred during the passage of dye in either direction.

5. Radial transfer occurred from early to late wood and from one annual ring to another.

6. Evidence is given showing that the passage of dyes occurred only in the vessels.

7. The radial and tangential anastomoses of vessels is demonstrated anatomically.

8. The rates of movement are recorded, showing a dependence on season, direction of movement, and method of application of the dye.

9. The rate of upward movement is shown to exceed the rate of downward movement when transpiration is rapid, but not when transpiration is stopped or slow. An explanation is suggested.

10. The relation of these results to previous results and theories is briefly discussed.

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TILE-CELLS IN THE RAYS OF
THE MALVALES

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(With Plate IX and 8 figures in the text)

I N an earlier paper (3) the writer called attention to the occurrence
of "tile-cells" in the rays of some members of the Sterculiaceae.
The name has been given to a peculiar type of ray structure which is
not confined to this family, but is found in some genera of the
Bombacaceae, Tiliaceae and Sterculiaceae, though it does not, as far
was we know, occur in any other group of plants. For this reason the
whole group of the Malvales has been studied rather than the Ster-
culiaceae alone.

The original German term "ziegelsteinförmig," from which our
term "tile-shaped" is derived, was given to cells which look like
Roman bricks on the radial section of the wood, because they are
extremely narrow radially. In some cases, for example in *Neesia
synandra* Mast., the tangential walls are so numerous that, although
they are very thin, they become the most conspicuous feature of the
radial sections of the wood.

References to tile-shaped cells occur in the standard works on
wood structure such as Moll and Janssonius (5), Record and Mell (6),
Solereder (7), etc.: but these writers are content with a mere state-
ment of the existence of these cells and make no attempt to explain
either how they arise or what relation they bear to other types of ray
cell. Even Forsaith (4), whose figures of *Durio zibethinus* D.C. show
these cells, makes no mention of them in his paper on "Some features
of the anatomy of the Malvales." Bargagli-Petrucci (1) mentions tile-
cells in his descriptions of woods from Borneo, but does not enter into
great detail, though he stresses the great difference between these
cells and the erect marginal cells of other heterogeneous rays. During
the past year an examination has been carried out which covers over
two thousand woods of different genera and families, and though
many different types of ray have been met with, even rays which have
no procumbent cells at all, as well as those with both procumbent and
erect cells, yet all are different from these rays of the Malvales, and

one is forced to the conclusion that the tile-cells of the rays of the Malvales represent a unique condition, confined to this group, though not universal within it, for they occur only in some genera of three of the families, Tiliaceae, Bombacaceae and Sterculiaceae.

The following definition of tile-cells is suggested: "Special type of erect cells, without visible contents, occurring in radial series, much narrower radially than the procumbent cells of the ray, and interspersed among them."
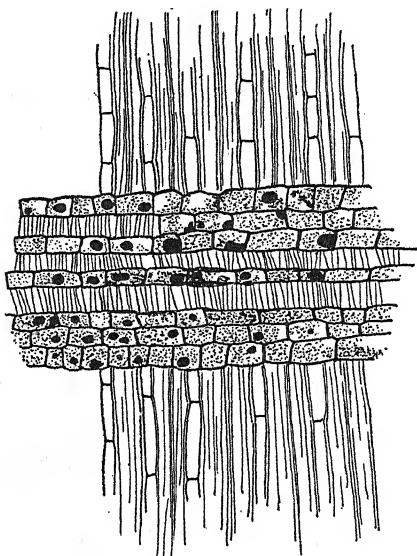
Text-fig. 1. Radial longitudinal section of the wood of *Boschia mansoni* Gamble, showing ray with tile-cells (× 75).

Two extreme forms of tile-cell can be distinguished, which will, for the sake of convenience, be called the *Durio* and the *Pterospermum* type.

In the *Durio* type, the tile-cells are a conspicuous feature of the transverse and radial sections of the wood, but they are not always distinguishable on the tangential section. *Coelostegia griffithii* Benth. shows this type clearly (Pl. IX, fig. 1). The tile-cells are no wider tangentially than the procumbent cells, but are much narrower radially, and about 10–14 correspond to one procumbent cell. On the tangential section the difference between the tile-cells and the procumbent cells is slight, and only the presence of dark contents makes the procumbent cells a little more noticeable. On the radial section

the narrower diameter of the cells is very clearly marked. Tile-cells of this type (Text-fig. 1) are found in the Sterculiaceae in *Guazuma*, *Kleinhofia*, *Leptonychia*, *Reevesia*, *Scaphopetalum* and *Triplochiton*; in the Tiliaceae in *Columbia*, *Luehea* and in some species of *Grewia*; and in the Bombacaceae in *Durio*, *Cullenia*, *Neesia*, *Boschia* and *Coelostegia*.

In the *Pterospermum* type the rays are composed of two kinds of cell which can be recognised on all sections, but on the transverse
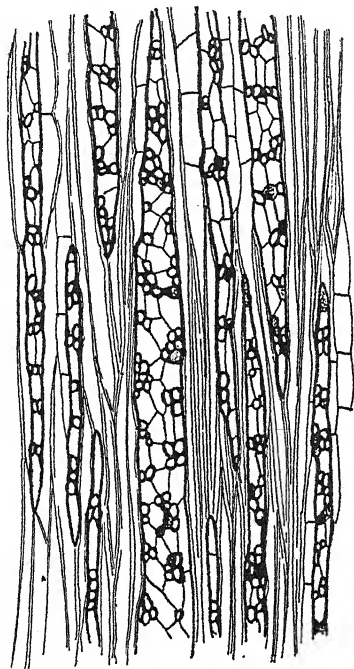
Text-fig. 2. Tangential longitudinal section of the wood of *Pterospermum acerifolium* Willd. showing ray with tile-cells ($\times 75$).

section the tile-cells do not form a conspicuous feature of the wood, and they might pass for ordinary marginal ray cells if only that section were considered. On the tangential section the rays are seen to consist of large angular cells which are devoid of contents, interspersed with clusters of smaller cells with dark contents (Text-fig. 2). The large cells are the tile-cells and the small cells are the procumbent cells of the ray. This is more clearly seen on the radial section, where the proportions of the cells can be made out (Text-fig. 3). The procumbent cells are usually about half as high vertically as the tile-cells and about

four to six times as long. The absence of contents, a characteristic feature of all tile-cells, adds to their distinctness, and they stand out sharply from the darker procumbent cells. Tile-cells of this type are found in the Sterculiaceae in *Pterospermum*; in the Tiliaceae in *Belotia*, *Duboscia* and some species of *Grewia*; and in the Bombacaceae in *Hampea* and *Ochroma*.
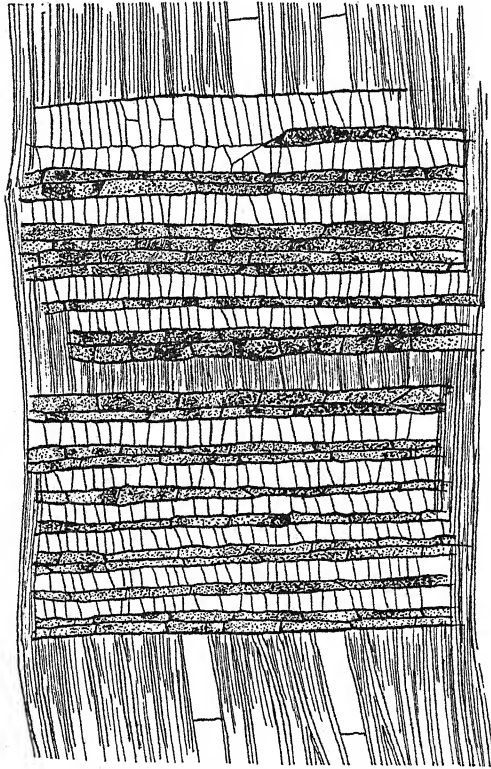
Text-fig. 3. Radial longitudinal section of the wood of *Pterospermum acerifolium* Willd. showing rays with tile-cells (× 75).

Moll and Janssonius [5] reserve the term "tile-cell" for the very narrow cells, and do not apply it to those of *Pterospermum*. But, in the author's opinion, the precise shape of the cells is less significant than their position in the centre of the ray, and on these grounds the cells of *Pterospermum* and *Durio* represent extreme forms of the same feature, and both should be regarded as "tile-cells." There is a complete sequence of intermediate forms, and the exclusion of the

Pterospermum end of the sequence must rest on a purely arbitrary division. On the other hand there is a clear distinction between tile-cells of both forms and ordinary upright ray cells, and both the *Pterospermum* and the *Durio* types may be found in the same genus. For example, in the genus *Grewia*, *G. microcos* Linn. is similar to *Durio*; *G. Rolfei* Merrill, *G. popolifolia* Warb. and *G. multiflora* Juss are like *Pterospermum*; while *G. stylocarpa* Warb. is a borderline type, and might be included in either. *Guazuma* and *Reevesia*, in
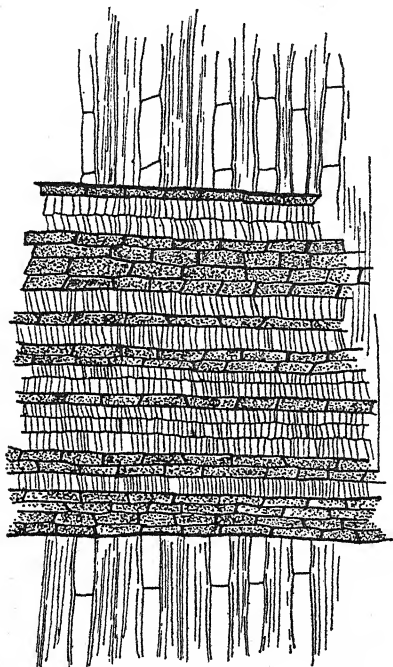
Text-fig. 4. Radial longitudinal section of *Guazuma ulmifolia* Lamk. showing ray with tile-cells which are only slightly higher than the procumbent cells (× 75).

which the development of tile-cells has been studied, are two intermediate forms; *Guazuma* (Text-fig. 4 and Pl. IX, fig. 2) has tile-cells which are very like those of *Durio* when they are seen on the radial section, but they are often slightly higher than the procumbent cells; this makes the two types of cell quite distinct on the tangential section, as in the *Pterospermum* type. *Reevesia* is very similar: the tile-cells are like those of *Pterospermum* when seen on the tangential section, but are closer together and narrower radially than is usual for that type.

Before discussing the origin of tile-cells it is necessary to consider their relation to the erect cells of other rays. All heterogeneous rays are composed of cells of more than one size and shape, and though the marginal cells sometimes differ very little in shape, size or contents from the procumbent cells, there is a common type of ray, which is found in many different families, which has a short middle part of procumbent cells and a wide margin of erect cells. The latter cells are often entirely devoid of contents and may appear on radial sections to be very similar to the rays of *Pterospermum*. *Corynanthe pachyceras* K. Schum. (Rubiaceae) shows the similarity on the radial, and the essential difference on the tangential section (Pl. IX, fig. 3 *a*, *b*). The erect cells of *Corynanthe* are really all marginal cells of heterogeneous rays; they may extend as long tails and occupy the greater part of the length of the ray, but they only occur as a uniseriate waist which apparently connects two multiseriate rays. Another less common type is shown by *Odontodenia speciosa* Benth. (Apocynaceae), where the rays, as seen on the tangential section, are very similar to the small rays of the *Pterospermum* type, but the small cells appear to be subdivisions of the erect cells, as they are seen to be square on the radial section, and are of the same radial diameter as the erect cells.

In considering the relations between tile-cells to the erect marginal cells of heterogeneous rays we find that tile-cells of the *Durio* type differ from erect cells of heterogeneous rays in contents, shape and position in the ray. But all of these points of difference are not always present, for the marginal cells of rays are often without contents, and the tile-cells of the *Pterospermum* type may approach very closely to erect marginal cells in shape, so that only the position in the ray remains as an indication of their nature; the erect cells of heterogeneous rays are marginal; tile-cells are central as well as marginal.

Another form of heterogeneous ray, which is less common, is the ray with a more or less continuous sheath of larger cells surrounding it. These are "Hüllzellen" or "sheath-cells," which are large, erect or square cells which form a more or less complete sheath round the central procumbent cells of a multiseriate ray. They are common in the Malvales generally, and may occur in woods with tile-cells. In most genera of the *Pterospermum* type the marginal cells are identical with the central ones and are undoubtedly tile-cells, but *Hampea panamensis* Standl. provides an interesting intermediate type with sheath-cells of all shapes from square to erect, and central tile-cells. Some of the rays have no tile-cells at all, but only a layer of sheath-cells, while others have central tile-cells very like those of *Ptero-*

spermum. In the *Durio* type the ray may be surrounded by a sheath of larger cells which are square to erect on the radial section as in *Leptonychia* and *Scaphopetalum,* but which differ from the extremely narrow tile-cells of this type.

Where large heterogeneous rays and uniseriate rays are present in the same wood, the cells of the uniseriate rays are usually like the marginal cells of the multiseriate rays and not like the central procumbent cells. This is most noticeable in rays with long tails of erect cells and short multiseriate parts of procumbent cells, as in *Corynanthe.* Here the uniseriate rays are all formed of erect cells. This finds a counterpart among the Malvales, where there are both multiseriate and uniseriate rays in the same wood. If the large rays have sheath cells, the uniseriate rays are composed entirely of large erect cells, as in *Leptonychia* and *Scaphopetalum.*

In other genera with tile-cells of the *Durio* type both the small rays and the multiseriate rays consist of alternating rows of procumbent and upright cells which cannot be distinguished easily on the tangential section, and the small rays are then uniseriate. But in the borderline types and the *Pterospermum* type, where the tile-cells are much higher and wider than the procumbent cells, true uniseriate rays do not occur, for the large cells alternate, not with single procumbent cells, but with small groups of them. Here again *Hampea panamensis* Standl. provides an interesting intermediate condition, as there are both true uniseriate rays, formed of large cells like the sheath cells, and also rays like the small rays of *Pterospermum* consisting of large tile-cells alternating with groups of small cells.

These rays suggest an hypothesis to account for the formation of the initials of heterogeneous rays from fusiform initials. Beijer [2] in his work on *Aeschynomene elaphroxylon* Guill and Perr. describes the formation of secondary uniseriate rays by the subdivision of fusiform initials by horizontal walls, and their subsequent development into multiseriate rays as the result of the formation of further horizontal and vertical walls. The small rays of the Malvales and the long-tailed heterogeneous rays cannot fail to suggest a similar development of their initials, and it is hoped to pursue this aspect of the investigation as soon as suitable material can be found.

The development of tile-cells from the cambium has been followed in *Guazuma tomentosa* H.B. and K., *Durio zibethinus* D.C. and *Reevesia thyrsoidea* Lindl. in material grown at the Royal Botanic Gardens, Kew. In fresh material of *Guazuma tomentosa* the tile-cells and procumbent cells of the rays can be clearly distinguished on all

sections by their different contents, as well as by differences in cell size. The tile-cells contain protoplasm and conspicuous nuclei only in the immediate neighbourhood of the cambium, and they lose their

cambium   ⟵ xylem ⟶
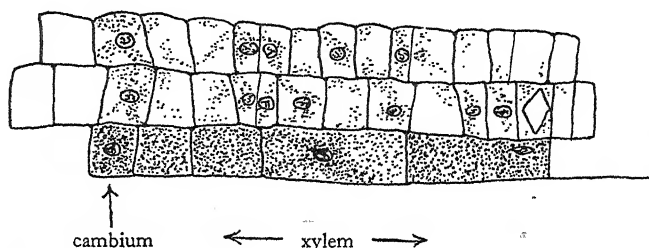
Text-fig. 5. Development of tile-cells in a ray of *Guazuma tomentosa* H.B. and K.

phloem   cambium   xylem
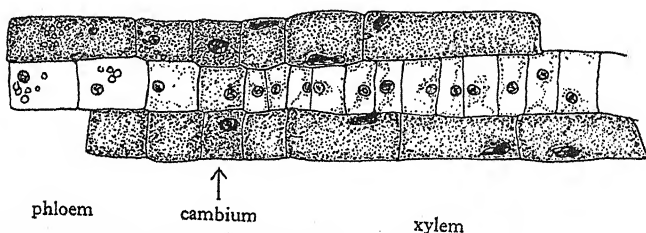
Text-fig. 6. Development of tile-cells in a ray of *Guazuma tomentosa* H.B. and K.

cambium   xylem
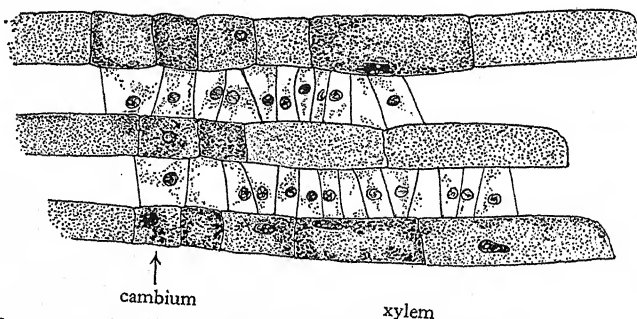
Text-fig. 7. Development of tile-cells in a ray of *Guazuma tomentosa* H.B. and K. Showing dividing cells which will become tile-cells and elongating procumbent cells.

contents at an early stage. The procumbent cells have dark contents, probably of a tannic nature, and these contents are present in all the procumbent cells, even those of the cambium itself. The cambial initials of the tile-cells and procumbent cells do not differ markedly

in size (though those of the tile-cells are sometimes slightly higher vertically), and the cells which are cut off from them towards the wood are at first very much the same size as the initials (Text-fig. 5). But while the cell which is to become a procumbent cell elongates radially, that which is to become a tile-cell divides to keep pace with this elongation (Text-fig. 7), and the subdivision is continued till the procumbent cell has attained its full length, by which time there may be eight to ten tile-cells corresponding to it. That this result is achieved by subdivision of the cell originally cut off from the cambium, and not by more frequent divisions of the cambial initial is clear from the accompanying text-figures which show one, two or more tile-cells corresponding to the elongating procumbent cell (Text-figs. 5, 6, and 7). If the cambial initial of the tile-cells had divided more rapidly than that of the procumbent cells the same final result might be attained, but there should already be eight to twelve extremely narrow cells formed, which should enlarge to keep pace with the elongation of the procumbent cell. This never occurs, while it is possible to obtain sections, both transverse and radial, which show different stages of subdivision of the tile-cells. The retention of the nuclei and protoplasmic contents of the tile-cells till this subdivision is completed corroborates this, as does also the thinness of the walls of the tile-cells at this stage. The nuclei and protoplasmic contents of the tile-cells are retained until the procumbent cells have reached their final size and maturity, then the walls thicken, the nuclei and protoplasm are gradually lost, and the cells are incapable of further subdivision. A diagrammatic representation of the development of a ray of this kind is shown in Text-fig. 8.

A similar course of events was observed in *Durio zibethinus* D.C., but in this plant the procumbent cells elongate less than in *Guazuma* and the different stages are passed through more quickly, and are consequently less easily observed; the inclusion of crystals in the tile-cells, and their consequent horizontal division, adds a further complication which makes this a less favourable plant for study.

The material of *Reevesia thyrsoidea* Lindl. was unfortunately much crushed in the region of the cambium, and it was not easy to follow the development of the tile-cells. It seems probable, however, that here too the development follows the same course as in *Guazuma*. The distinction between the procumbent cells and the tile-cells is less marked than in the two preceding plants, as the tile-cells contain starch instead of dark contents. Staining with iodine shows clearly the difference in contents between the two types of cell.

There are several points which have been noticed during this study of developmental stages which are worthy of further consideration, for though their significance is not yet fully appreciated they suggest ideas that may lead eventually to a clearer understanding of the nature of tile-cells.

First, tile-cells are confined to that part of the ray which traverses the xylem. In all fresh material examined the cell rows were continued outwards through the phloem as large parenchymatous cells which bore no resemblance to the narrow erect cells of the wood.

xylem

↑
cambium
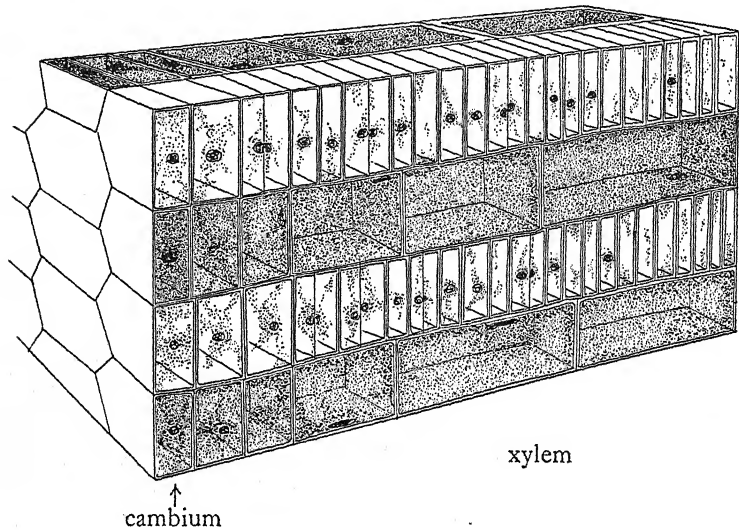
Text-fig. 8. Diagrammatic representation of the development of
a ray with tile-cells.

They were constantly filled with starch grains, but never with the dark contents which form such a conspicuous feature of the procumbent cells in both wood and phloem.

A second point of great interest is the distinction between the initials of the two types of cell. The cambial cells which give rise to the rows of procumbent cells are clearly marked out by their dark contents, while those of the tile-cells appear transparent, containing only protoplasm and nuclei. In *Durio* and some other genera the tile-cells constantly contain crystals, and these may appear in the cell almost immediately after its formation from the cambium. Frequently two or more crystals are formed in a single tile-cell, usually after

horizontal divisions have formed almost isodiametric daughter cells. Such cells apparently lose their protoplasm and nuclei at a very early stage and do not undergo any further subdivision.

In woods which, unlike the *Pterospermum* type, have little distinction in shape between the initials of the different cell rows, the formation of the tile-cells or procumbent cells appears to depend on the presence or absence of dense contents. A good example of this is to be found in *Durio*. Although it is difficult to follow a single cell row with certainty through any great distance in the wood, yet single cells can be followed sufficiently far in thin radial sections to show that a change may take place, though infrequently, from tile-cell row to procumbent cell row, and *vice versa*. The change is always accompanied by a change in the contents of the cell, the procumbent cells having always the characteristic brown contents, and the tile-cells crystals or no contents except slight remnants of protoplasm.

The formation of tile-cells depends therefore on the capacity of the daughter cells of the cambium to elongate or subdivide, and the fact that this in its turn appears to depend on the presence or absence of definite cell contents, raises questions of osmotic and physical relationships within the cell that come within the province of the physiologist rather than the wood anatomist.

The study of *Guazuma*, which is intermediate between the two types of tile-cell, suggests that the development must be the same in both types, but though the absence of dark contents from the tile-cells is such a characteristic feature, it cannot be the only factor involved, for the cambial initials of the two types of cell also differ fundamentally in size and shape, and the two types cannot interchange as in *Durio*. In *Guazuma*, where the difference between the two types of initial is so small, there appears to be no interchange between the cell rows.

The frequent absence of contents from the erect marginal cells of heterogeneous rays is of interest in this connection, for here too it is conceivable that the deciding factor as to whether the cell elongates or subdivides may be the presence or absence of contents. In woods like *Corynanthe*, where the marginal cells are of greater height than the inner ones as well as of less radial diameter, the conditions may be similar to those in *Pterospermum*, and the formation of the erect cells may depend on the interaction of at least two factors.

In conclusion the author would like to suggest that the shape of the initials of heterogeneous rays, including especially those rays of the Malvales which have tile-cells, depends on the amount of subdivision

of the fusiform initial, but that the ultimate shape of the mature cells depends not only on the shape of the cambial initial, but also on a physiological factor within the cell which causes some cells to elongate radially after they are cut off from the cambium, and others to sub-divide.

Summary

1. The name "tile-cell" has been given to certain ray cells which have an extremely narrow radial diameter, and which differ also in their lack of contents from the procumbent cells of the ray.

2. They are found in some genera of the Malvales, but have not been recorded in any other group of plants.

3. Two types of tile-cell occur, the *Durio* and the *Pterospermum* type, which are quite different from one another in extreme examples, but are linked by certain genera such as *Grewia*, which contains both types.

4. Tile-cells differ from other erect ray cells in three points—their shape, their lack of contents and their position in the ray.

5. Of these the third is the most constant, the erect cells of hetero-geneous rays may be somewhat similar to tile-cells in shape, and may be without contents, but they are marginal, while tile-cells are central as well as marginal.

6. The development of tile-cells from the cambium has been followed in fresh material of *Guazuma tomentosa* H.B. and K., *Durio zibethinus* D.C. and *Reevesia thyrsoidea* Lindl.

7. Tile-cells are confined to that part of the ray which traverses the xylem; the initials of the two types of cell differ markedly in contents, the initials of the procumbent cells having dark contents, those of the tile-cells only protoplasm and nuclei.

8. The daughter cell of the initial of a procumbent cell elongates after its formation from the cambium, while that of a tile-cell sub-divides to keep pace with this elongation.

9. It is suggested that the formation of tile-cells depends on the capacity of the daughter cells of the cambium to elongate or sub-divide, and that this in its turn may depend on the presence or absence of certain cell contents.
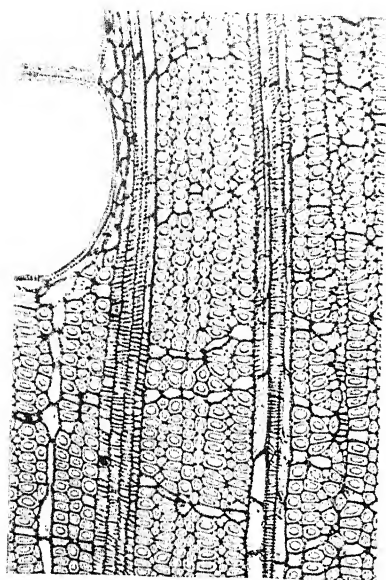
Fig. 1   Fig. 3 *a*   Fig. 3 *b*

Fig. 2   Fig. 4 *a*   Fig. 4 *b*

CHATTAWAY—TILE-CELLS IN THE RAYS OF THE MALVALES

(× 100)
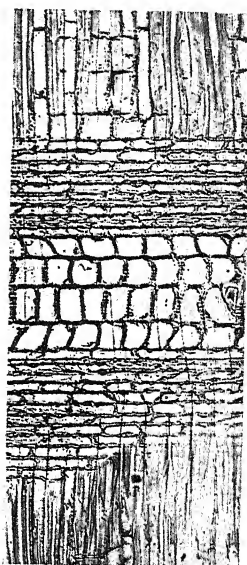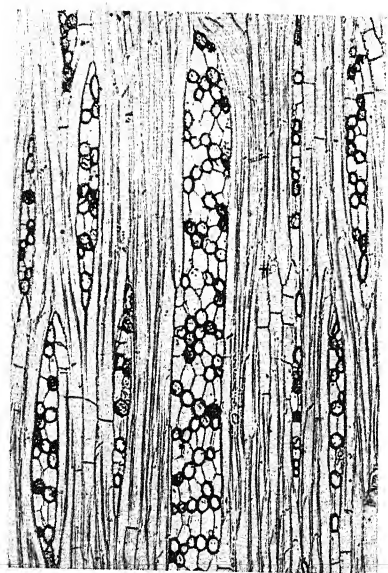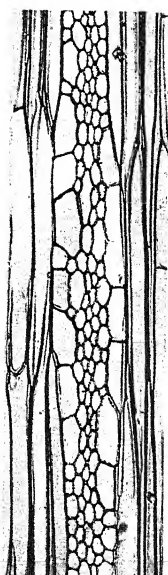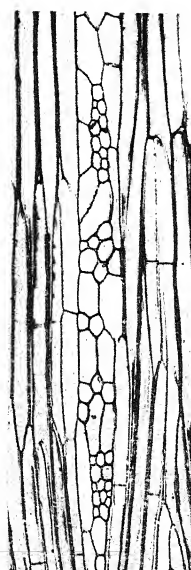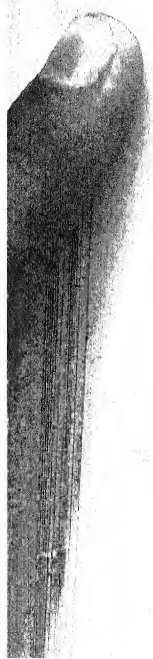
the investigation; to Dr J. Burtt Davy, who kindly checked the botanical names of the specimens; to the Director of the Royal Botanic Gardens, Kew, for fresh material; and to the following from whom wood specimens have been received: Prof. G. Bredeman, Institut für angewandte Botanik, Hamburg; Prof. A. Chevalier, Musée d'Histoire Naturelle, Paris; Dr F. W. Foxworthy, Forest Research Institute, Kepong; Prof. H. H. Janssonius, Kolonial Institute, Amsterdam; Prof. S. J. Record, Yale School of Forestry; Dr L. J. Reyes, Bureau of Forestry, Manila; the President, Forest Research Institute, Dehra Dun; the Director, Forest Products Research Laboratory, Princes Risborough.

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EXPLANATION OF PLATE IX

Fig. 1. Transverse section of the wood of *Coelostegia griffithii* Benth. showing rays with tile-cells.

Fig. 2. Longitudinal tangential section of the wood of *Guazuma ulmifolia* Lamk. showing thin-walled empty tile-cells, and thicker-walled procumbent cells; the latter often have dark contents.

Fig. 3. *a*, longitudinal tangential; *b*, longitudinal radial section of *Corynanthe paniculata* Welw. (Rubiaceae); showing erect and procumbent cells: the erect cells form a uniseriate waist between the two multiseriate parts of the ray.

Fig. 4. Longitudinal tangential sections of *Hampea panamensis* Standl. showing rays with *a*, sheath cells, and *b*, tile-cells.

(All × 100.)

SOME PHYSIOLOGICAL ASPECTS OF DIFFERENTIATION

By D. THODAY

I. Physiological diversity

For the study of plant metabolism the unit must ultimately be the cell. It is, however, only in special cases that an approximation to this ideal subject can be used in experimental researches. Plant physiologists have dealt predominantly with organs, and must very largely continue to do so, for practical reasons, or of necessity.

Organs, especially those of higher plants, are of complex constitution. Much attention has been devoted to the physical consequences of their structural elaboration, especially in the case of the foliage leaf. On the other hand, there is room for doubt whether the possibilities of *physiological* complexity have received as adequate recognition.

The various structural elements, with different physiological functions, are so intermingled in the organs of plants that separation of them for macrochemical analysis is impracticable. Doubt, therefore, attaches to the interpretation of the macrochemical data in the absence of adequate microchemical methods with which to supplement them. This difficulty has been encountered, for example, in attempts to discover the first carbohydrate of photosynthesis and follow the changes it undergoes. The assimilating tissues in a leaf cannot be separated from the conducting tissues, nor bundles from parenchyma in petiole or veins.

Macrochemical methods have, on the other hand, provided abundant evidence of differences in metabolism between similar organs even in closely related plants, and between different organs of the same plant, even such as are made up of similar tissues with morphologically indistinguishable elements.

Metabolic differences between similar tissues. An interesting example of a difference between allied species is afforded by *Helianthus annuus* and *H. tuberosus*. In the latter, inulin is found in the stem, as well as in abundance in the tuber, but not in *H. annuus*. Grafts between the two species made by Daniel [5] and by Colin [3] have shown

that carbohydrate transport occurs freely from one to the other, whichever is the scion, but each nevertheless retains its normal characteristics. This was particularly clear in a double graft in which a piece of sunflower was interpolated between the basal and apical parts of an artichoke: in the latter the basal parts, nourished *via* sunflower stem, stored abundant inulin, and the upper part also contained a normal amount of inulin, while the intervening sunflower stem contained none. Thus there exist between the parenchymatous cells of the two stems specific differences in metabolism. In the grafts the transition from one to the other according to Colin is sharp.

Similar differences are shown between different organs of the same plant. In both species of *Helianthus* the leaves form starch as a temporary reserve carbohydrate during carbon assimilation; but in the stem it is found only in the starch sheath. In *H. tuberosus* no inulin is formed in the leaves (3). Thus even the green parenchymatous cells of the cortex differ physiologically in a marked degree from the cells of the leaf mesophyll, and even from those of the petiole.

Similar differences between different organs are described by Ruhland and Wetzel (25) for rhubarb. The rhizome contains very little acid and its nitrogen is largely present in soluble form, chiefly as amino-acid and to a less extent as amide, with very little ammonia. In the petiole, ammonia forms two-thirds of the total nitrogen, and acid is abundant—both formed, according to Ruhland and Wetzel, by deamination of amino-acids translocated from the rhizome. In the blade, the bulk of the nitrogen is present as protein, which is synthesised from ammonia coming from the petiole; there is little ammonia and only a trace of amide. These profound differences exist although the bulk of all these organs consists of parenchyma, and they do not, it seems, merely represent stages in a cycle of development and senescence. The well-known differences between storage and other organs revealed by elementary anatomical and microchemical examination provide abundant evidence of the same kind.

Metabolic diversity within an organ or a tissue. Within a single organ, the elements constituting the principal tissues have distinctive characteristics, not only of form but of constitution and content, which we associate with their performance of different functions.

It is not, however, between the chief classes of tissue element only that physiological differences are found. There are, for example, in otherwise apparently uniform tissues, various peculiar cells which are labelled idioblasts. Their physiological properties are sometimes,

perhaps commonly, dismissed from further consideration as idio-syncratic. Whatever view is taken of their significance, it is clear that their metabolism must differ distinctly, if not fundamentally, from that of the cells around them.

Some of the facts are commonplaces of plant anatomy. Such are the localisation, in particular cells or cell groups, of calcium oxalate as raphides or sphaerocrystals, of tannins, or of anthocyanins.

The localisation of other substances requires special tests for its demonstration; but illustrations are not uncommon in text-books of plant microchemistry, although in general the facts have been accumulated incidentally.

Some of the phenomena relating to sap solutes are of an astonish-ing character and present difficult problems.

Selective accumulation of solutes. Schimper (28), in the course of his investigations on the formation and significance of calcium oxalate in relation to the assimilation of nitrate, sulphate and phosphate in leaves, examined the distribution of nitrate in particular. His obser-vations, on the whole, are what might be expected if nitrate passes through the plant from the soil to be built up into organic nitrogen in the mesophyll of the leaves; for in general he found the concentration of nitrate diminishing from the petiole to the minor veins and thence to the mesophyll. In some plants, however, which were much richer in nitrate than others, Schimper records that the epidermis gave a stronger reaction even than the parenchyma of the veins. Thus nitrate accumulates in the epidermis in spite of its utilisation in the assimilating tissues. It is held there against a concentration gradient.

Schimper also states that the bundles themselves showed little nitrate in most cases, from which it must be inferred that nitrate is accumulated as such in adjacent parenchyma. Hairs on the leaves of potato, *Chenopodium Bonus-Henricus* and *Hyoscyamus niger* gave a stronger reaction than adjacent epidermal cells. Schimper also records the accumulation of phosphate in hairs of *Ageratum*.

It is difficult to regard such facts merely as features of a dynamic equilibrium between accumulation by transpiration on the one hand, and utilisation in the assimilating tissue on the other, of a readily diffusible substance. Still more difficult is Molisch's observation that the pith of *Helianthus* accumulates nitrate often to such an extent that when dry it deflagrates (19).

Giessler (7), and later Patschovsky (21), studied the distribution of oxalic acid and tannin in a large number of plants from the point of view of the utility of the substances as "Schutzmittel." Besides

giving additional information regarding the occurrence of tannin, they recorded very interesting examples of localisation of soluble oxalate.

In leaves of *Rumex acetosa* Giessler found oxalate most abundant in the epidermis, but the guard cells of the stomata contained none, and the epidermal cells adjacent to them little or none. Vascular bundles in leaf and petiole were free from oxalate, as was also the case in *Oxalis* and *Begonia*.

Among Patschovsky's more extensive observations some of the most interesting from the present point of view apply to succulents. His results for a number of species of *Mesembryanthemum* give the general impression, that cells of certain well-marked types, characterised respectively by storage of oxalate in solution, of calcium oxalate raphides and of tannin, are of very general occurrence in various proportions, and represent units, with distinctive functional activities, which are more or less characteristic of the genus.

Recent work in this laboratory (32) has added the fact that cells containing calcium malate in solution are commonly present in the water tissue of *Mesembryanthemum* leaves, in proportions varying with the species, side by side with cells containing soluble oxalate. Macrochemical analysis can, obviously, give only very inadequate information regarding such a system.

A special study has also been made of the succulent composite *Kleinia articulata* (32). In the stem of this plant calcium malate is localised in the pith and part of the inner cortex. Phosphate also shows strong localisation in the bundle zone and, if more abundant, in the outer cortex. The cells of the hypodermal collenchyma contain rhomboidal crystals of calcium oxalate. Inulin is stored in the bundle zone. Other inorganic ions also are unequally distributed, though exact determination of their distribution is not in all cases possible. Physicochemical problems are clearly involved. We have to recognise, however, as the primary aspect of the phenomena, that the physiological activity of the cells must differ, in some cases sharply, from one region of the stem to another, even though the cells appear indistinguishable in structure.

Association of metabolic with morphological differences. There are other cases in which differences in physiological activity cannot without further consideration be taken as evidence of physiological differentiation. Some differences may be conditioned by the immediate environment of the cells. For example, in an ordinary dorsiventral foliage leaf, palisade and spongy parenchyma are differently situated as regards illumination and carbon dioxide supply.

On the other hand there are many foliage leaves in which physiological differentiation is evident, in association with morphological differentiation.

In the bifacial leaves of species of *Metrosideros*, for example, the paliside tissue is sharply differentiated from the large-celled colourless parenchyma within. In the latter (in *M*. ? *rigidus*) most of the cells store starch in larger grains than occur in the palisade cells; scattered amongst the starch-bearing cells are cells containing spherocrystals of calcium oxalate, with little starch in smaller grains. Here we have to deal with differentiation of the sort described for the succulent leaves of *Mesembryanthemum*. Similar examples could be multiplied.

The leaf of *Peperomia tithymaloides* is specially remarkable (32). A thick layer of water tissue of large cells covers the upper side. Even within this layer there is differentiation in respect of calcium and phosphate content of the sap. Next below comes a single layer of small palisade cells, each with a sphaerocrystal of calcium oxalate; minute starch grains occur in the chloroplasts. The bulk of the spongy parenchyma stores large starch grains in abundance; but near the lower epidermis the grains are small and a shallow hypodermal zone of cells contains soluble calcium.

Cell structure as a result of physiological activity. In the various examples that have been given, some of the differences in content are the cumulative result of past activity.

More familiar cumulative differences are those which manifest themselves as differences of form and structure. These also are the results of different modes of physiological activity during development.

The strut cells of a *Hakea* leaf, for example, develop their characteristic form and their thick lignified walls through metabolic processes which differ from those occurring in the adjacent assimilating cells. The accumulated products of metabolism are in this case colloidal solids, in other cases they may be crystalline compounds, or soluble substances like malic acid, that remain in solution in the sap. The phenomena may or may not have a morphological aspect, but they are all from their inception essentially physiological.

It follows that any attempt to understand how physiological differences arise cannot logically be confined to the kind of phenomena with which we have been primarily concerned. The inquiry inevitably extends to cover differentiation as a whole.

II. The physiology of differentiation

Points of view. Facts of the sort illustrated above have been accumulated by workers with very different aims and modes of approach. Schimper's object (28) was to throw light upon a particular phase of metabolism, the assimilation of nitrate, sulphate and phosphate. Many contributors have been occupied in extending the data of comparative or systematic anatomy. Physiological anatomists have aimed at elucidating the functioning of organs in relation to their structure and have interpreted the facts largely as illustrations of the principle of the division of labour.

Giessler (7) and Patschovsky (21) started out with a definitely teleological point of view, expecting to find the distribution of oxalic acid and of tannin correspond to the function of protecting the plant by making it distasteful. They did indeed find on the whole that the occurrence of these substances was more or less peripheral, and more or less complementary.

The modern attitude, however, rejects a frankly teleological approach. That avenue has led to much interesting descriptive work and the revelation of remarkable correlations, but it raises problems without contributing much to their solution.

Even the concept of division of labour savours of teleology. The fact of functional diversity is obvious, and interdependence is of course admitted. The concept is useful in dealing with the working of mature structures as mechanisms already in being. Some examples, however, raise the question whether "division of labour" is quite the right conception. Rather, they suggest a system of diverse units in "functional harmony," which is not necessarily the same thing. Wherein lie the functional advantages or necessity of tannin sacs, or of localised oxalic acid production?

The causal viewpoint. If we assume that the metabolic and structural differences between species are bound up with specific differences in chemical constitution we may suppose that these involve the formation of particular bye-products, some of them permanent and excretory, others temporary and mobilisable. Each individual plant achieves a solution of its own metabolic problem—each, as a complex of diverse units working together in harmony, represents a *modus vivendi*.

How the solution is achieved becomes from this point of view the centre of interest. How harmony is maintained is an aspect of the problem, which presents itself at every stage.

The physiology of differentiation so envisaged is of course a very wide as well as difficult subject, and a comprehensive treatment of it would be outside the scope of this contribution even if it were within the power of the writer or indeed of anyone in the present stage of knowledge. All that can be attempted here is to refer briefly to some aspects.

Differences of kind or of degree? In the first place some further consideration of the fundamental nature of the differences that are observed between cells may not be out of place.

Consider first the carbohydrate relations of the spongy and palisade tissues of leaves. It is natural at first sight to attribute the differences in starch content to the influence of different conditions incident upon the cells in different parts of the mesophyll. It was primarily in order to avoid *physical* complications of this sort that Warburg [36] and others chose for investigations on photosynthesis a unicellular alga. On the other hand, there is little justification for an assumption that the mesophyll is *physiologically* homogeneous. In cases where, as observed by Langner and others [16], the spongy tissue continues to increase its starch content in the evening after the palisade cells have begun to lose their starch, a difference of the critical concentration of sugar for starch formation seems to be indicated.

A difference of this kind can be regarded as of a quantitative nature. The critical concentration is certainly variable in any one cell, for in a wilted leaf starch dissolution proceeds more rapidly than in a turgid one [20].

Nevertheless, we are not in a position to relate the differences in critical concentration between different cells of the mesophyll to the immediate effects of conditions incident upon the individual cells.

On the other hand, the guard cells of stomata can switch over from starch formation to starch dissolution under the influence of external stimuli. Using this analogy, the differences between cells in different parts of the mesophyll might be regarded as primarily differences of behaviour of a correlative character, induced by conditions associated with their position in the leaf. In this connection the outcome of Scarth's attempt to explain stomatal movements as direct effects of external conditions, not induced responses, will be awaited with interest [27].

Differences of a similar kind, in that they indicate changes in critical concentrations and metabolic balance, mark the stages of development of tissues from the growing point, as recorded by Sachs [26]

and de Vries(6)*; but here they follow a regular sequence in time, relatively independent of external conditions, working out a cycle of activity, the causes of which are inherent in the organ itself.

Turn now to examples like the leaf of *Metrosideros*, with clear structural and physiological divergence between assimilating and storage tissues, or of *Mesembryanthemum* with several distinct physiological types of cells. Here the differences seem to be definitely qualitative. They look as though the divergences in development have been responses of an all or nothing type.

Since,.however, all the different kinds of elements are derived from the same embryonic type by a continuous process of development it seems necessary to suppose that differentiation at its inception is quantitative, or rather that it is initiated by way of quantitative changes in the cell metabolism. Nevertheless they result sooner or later in profound differences, usually of structure as well as in functioning. These are largely irreversible, though in regeneration, or in normal formation of secondary meristems, the protoplasm may revert to the type of metabolism characteristic of embryonic tissues.

How is the inception of differentiation to be conceived? Two possibilities suggest themselves. One is that the differentiating cells, under the influence of stimuli correlated with their relative position, adopt at an early stage their particular rôle. The other is that a combination of mutual and external influences is continuously at work determining more or less directly the observed progressive divergence. Probably both kinds of determination will be found to play a part.

Mutual influences. In general, both the requirements and the metabolic products of different kinds of cells must differ. In a harmonious system, requirements must on the whole be complementary. Both demands and products are likely to result in mutual influence. Such mutual influence and interdependence of one cell or tissue upon another must play a very important part during development.

Küster(13) has collected together many examples of correlations which he interprets as results of mutual or one-sided influence, such as the association of anthocyanin or its absence with veins, hairs, or stomata, and of wood parenchyma or other elements with vessels. Jost(11) has similarly interpreted the correlation of respiratory cavities with stomata.

* The writer acknowledges his indebtedness to Dr F. F. Blackman for the references to de Vries' work as well as, directly or indirectly, for the general basis of interpretation of it.

The demonstration of influence is difficult. Yet such correlations must have a mechanism. Küster considers various kinds of influence which may be concerned. Mechanical constraint and accommodations both active and passive must play a considerable part in the development of the mature form and arrangement of cells. Many histogenetic processes, on the other hand, afford examples of adjoining cells assuming similar or identical forms and so adding to a tissue complex, as if the particular mode of development were contagious: one of the best examples is the completion of a broken ring of pericycle fibres by stone cells.

Correlations in the vascular system. Küster points out also that the formation of vascular bridges, in regeneration, between existing strands is evidence of some kind of influence emanating from these strands. The connecting up of severed strands in Vöchting's transplantation experiments [35] demonstrated a polarity in the vascular strands to which the course of the connecting strands conformed. Similar phenomena were observed by Janse [8] in experiments with *Helianthus annuus* (cf. also La Rivière [17]).

To Simon [29] we owe a demonstration that new vascular connections start from the *basal* ends of severed strands and grow from these points towards upper severed ends or, if these are not within reach, to other unsevered bundles. Here the primary phenomenon is apparently the formation of xylem elements. Cambial activity follows. In some of Simon's experiments, young stems of *Achyranthus* and *Iresine* were cut across and put together again with a perforated mica slip between, so that they could reunite only in the middle in the pith, and the vascular connections had to pass through this narrow parenchymatous union. Some directive influence is obviously at work in such cases. Simon favoured the hypothesis that it is a gradient of water deficit. Janse [8] suggested the diffusion of a stimulating substance. In a recent paper Simon also speaks of stimulating substances in considering the interpretation of some remarkable grafts of two quite unrelated plants, *Solanum melongena* on *Iresine Lindeni* [30].

For the basipetal propagation of cambial activity from the buds in trees Priestley has recently collected together much evidence [23]. Parallel with this is the dependence of differentiation of vascular tissue on continuity with developing leaves, shown in Jost's classical experiments with *Phaseolus* seedlings [9,10] and inferred by the writer from the study of *Helianthus annuus* [31], and by Alexandrov in interpreting the structure of the root of the sugar-beet [1].

In *H. annuus* (31) there also appeared to be a later upward propagation of secondary xylem formation in the upper part of old leaf traces. Jost (12) has recently found evidence in the regeneration of decapitated roots of an influence of the part already differentiated on the structure of the regenerated part, for after regeneration some xylem strands were found to be continuous right through. He infers that the continuity of vascular strands in the intact root depends upon such an influence, and he discusses possible mechanisms.

Cells are active units. However these correlations are viewed and whatever influences are postulated to explain them, the units acted upon are living cells and their response is active and characteristic.

Even the most thoroughgoing and plausible attempt to explain a feature of differentiation as the direct result of the action of physical and chemical conditions, that of Lee and Priestley in relation to the cuticle (18), fails on close examination and only serves to strengthen the point of view here adopted. Lee and Priestley point to the prevalence of fatty substances in growing tissues, to evidence of fatty acids in the cell walls, to the permeability of the outer wall of the young epidermis to water and its exposure to the drying action of the atmosphere and to oxygen. They then picture the evaporation at the surface as causing a mass movement of sap towards the surface, carrying with it fatty acids, which condense and oxidise to form a skin, like that which forms on the surface of a drying oil.

It is not necessary to consider here whether this suggestive analogy gives a true picture of the chemistry of cuticle formation. What does bear upon our problem is the assumption that the formation of cuticle is, so to speak, the automatic result of the exposure of the surface; the materials flow towards the surface from the underlying tissues generally.

Otto Damm (4) described phenomena in *Viscum album* which are very difficult to fit in with Lee and Priestley's view. In this plant, when the epidermis of the stem begins to crack under the strain to which growth in thickness exposes it, cuticular material appears outside subjacent cortical cells, mostly underneath the cells of the epidermis, except where the actual cracks appear. Several layers of such cells may thus form cuticle, one below another. Damm calls such layers "cuticular epithelium," implying quite justifiably that the cells in question are themselves the active source of the material of the cuticle.

An even clearer case is that of the so-called "collenchyma" of the fruit of the little mistletoe, *Arceuthobium pusillum* (33). This tissue is

several cells deep and practically without air spaces. Cuticular material is laid down within the walls, between inner cellulose layers of adjacent cells, as it were inflating the walls, except in places where the wall remains thin and "pits" are maintained. Though the resulting appearance is very collenchymatous the mode of formation is quite unique.

Whatever the process of maturation and hardening of this cuticular material may be, its initial formation can only be attributed to the secretory activity of the cells of the tissue itself, which is sharply delimited from the parenchymatous tissue within. This being so, the superficial cuticle which is identical in properties must likewise be regarded as secreted by the epidermis.

The ordinary epidermis is therefore a cuticular epithelium. The formation of cuticle depends primarily on the distinctive physiological activity of the epidermal cells. The presence of dry air at the surface may condition the maturation of the cuticle (though the examples quoted throw some doubt upon its necessity); there is no lack of evidence that it influences both secretion and maturation quantitatively; but it does not *determine* cuticle formation in the way Lee and Priestley pictured.

Determining and conditioning factors. The activity of the epidermis as a cuticular epithelium is characteristic and, with very few exceptions, such as those mentioned, is not paralleled by any but superficial cells. Yet those exceptions prove that the conditions associated with exposure at the surface cannot act as direct causes of the processes in question. Whatever may be the factor, correlated with the superficial position, which determines the specific activity of the epidermis, *it acts by inducing the cells to assume that rôle.*

A similar argument applies to the endodermis. Bond's observations on *Piper* stems (2) are sufficient to show that the analogous hypothesis proposed by Priestley and North (24) cannot explain the formation of the Casparian strip.

Physico-chemical studies have of course an important part to play. They throw light upon the mode of activity of cells, and on the ways in which various external and internal factors condition their activity. On the other hand, the explanation of *how* a determining factor induces a cell to assume a particular rôle is not likely to be soon achieved and attempts must be critically examined, for they are easily apt to involve over-simplification of the problem.

If we knew enough about the inner constitution and working of the cell we might be able to follow through the whole sequence of

cause and effect, but we do not. Our observations may establish correlations; but between the external agent and the result that follows is the internal agent, only partially understood, to the action of which the result is more directly due, and the mediation of which obscures the relation between the external agent and the resulting effect.

Directions of progress. Meanwhile there are two other main directions, besides the direct application of physico-chemical knowledge, in which progress may be hoped for. One is the intensive study of normal tissue correlations; the other is the study of deviations from the normal occurring naturally, or experimentally induced. Both may yield clues to the nature of the determining or inducing and the conditioning factors.

Contributory to these studies is a characterisation of the metabolic features of the different tissue elements and their classification accordingly, using functional and structural features as ancillary evidence.

There has been pioneer work in some directions; systematisation has also been attempted in different ways by Küster (14), by Ungerer (34), and by Pfeiffer (22). Much, however, remains to be done.

An interesting recent development is the comparative study of the protoplasm of cells in different plants and in different tissues, and of different cells in the same tissue. Weber has called it "protoplasmic anatomy." It promises, by the application of plasmolytic methods, vital staining, etc., to contribute valuable data on the permeability and other properties of different protoplasts. It has the merit that it concentrates attention on the living, active part of the cell, in contrast to the old anatomy which Weber stigmatises as the investigation of cell corpses! In this criticism there may be some truth; but structural detail, as already said, is the product of past activity, and is therefore to be valued as additional evidence of the distinctive characters of this activity.

Sooner or later the causal point of view must necessitate a reconsideration of comparative and phylogenetic as well as developmental morphology, implementing suggestions put forward by Lang (15) as long ago as 1915. These morphological bearings are, however, outside the scope of this contribution.

SUMMARY

Attention is drawn to the physiological diversity that exists among cells of similar morphology, in closely allied species, in different organs of the same plant and even within the same organ. The nature

of these differences is discussed. Some are graded and apparently quantitative, others clearly qualitative and sharp. The parallel between the latter and morphological differentiation is emphasised. Both come about through physiological divergence between equipotential embryonic cells taking their origin from the growing point.

Suggestions for the interpretation of the correlations exhibited in the arrangement of different kinds of elements are briefly reviewed. The conclusion is reached that external and mutual influences work largely as stimuli (or cues) inducing the cells, which are active not passive units, to adopt appropriate rôles from a limited repertoire.

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THE NATURE OF THE CAMBIAL STIMULUS

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(With 3 figures in the text)

JOST (1891 and 1893) discovered that leaves, and especially growing leaves, exert an influence that strongly promotes the growth of the cambium beneath them, that they do so even in the dark, and that their influence—the "cambial stimulus" as it will be called—travels only in the morphologically downward direction. His most critical experiment has been described in this journal by Priestley (1930): it was performed on seedlings grown from the start in the dark. The fact that the cambial stimulus travels only downwards (or obliquely downwards), even when the nutritive materials are travelling upwards, has been confirmed in different ways by Münch (1932, pp. 415–17) and by the writer (1932 a, p. 100).

It was suggested by Kastens (1924) that the cambial stimulus is a hormone, but no conclusive evidence has been offered to show that this is so. The evidence that comes nearest to showing that the cambial stimulus is a hormone is provided by some interesting experiments by Simon (1930). He succeeded in grafting together two plants of widely different families, and observed that new conducting strands began to differentiate on each side of the surface of contact and to grow towards one another even before the tissues of the two plants had grown together. But it is not certain that the stimulus which causes conducting strands to differentiate is the same as the cambial stimulus. Also the evidence is a little indefinite, and the experiments would be difficult to repeat.

The purpose of the experiments to be reported here was to test whether the cambial stimulus can cross a protoplasmic discontinuity. The tissues that were brought into contact belonged to two plants of widely different families, since it was thought that their protoplasms would be much less likely to unite than those of plants of the same species. But whereas in Simon's experiments one of the plants was placed in the position of a graft upon the other, in the present experiments both plants were kept on their own roots. The species used were pea and sunflower, and the arrangement was the following. Seeds

of pea and sunflower were sown in pairs close together, the peas being sown a few days the earlier. The pairs of seedlings were operated upon when the peas had expanded their fifth leaves, and when in the sunflowers the cotyledons were nearly full-grown and the first pair of leaves was from 10 to 20 mm. long (in two plants rather less).

In the peas, one of the mature internodes was split longitudinally, in the plane of the leaves, and one of the split halves was then cut through at the base, so that if formed a downward-pointing strip (see Fig. 1). In the sunflowers, the lower part of the hypocotyl was split
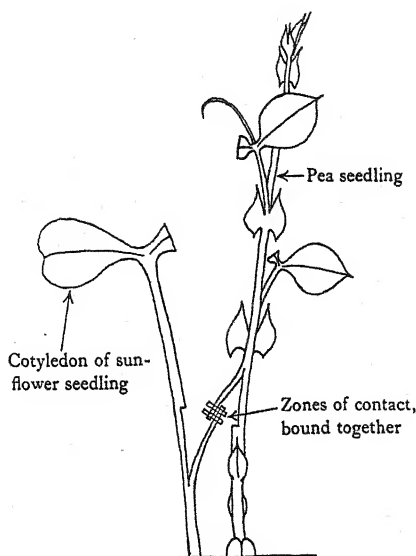
Fig. 1

longitudinally, and one of the split halves was cut through at the top, so that it formed an upward-pointing strip. The cut strips of the two plants were bathed with water, to wash away wound substances, and they were then tied with the end-parts of their longitudinal cut surfaces firmly in contact, in the manner shown in Fig. 1. The young shoots of the sunflowers were cut off close above the cotyledons, and any buds that began to grow from the axils of the cotyledons were continually removed. Vaseline was spread over all exposed cut surfaces, and over the outer sides of the zones that were tied together. These zones were from 2 to 5 mm. long, and the parts of the sunflower strips below them were from 8 to 15 mm. long.

As controls, other sunflower seedlings were operated upon in the same way and at the same stage, but were then left alone and not tied to other plants.

The next step was to examine, after a time, the upward-pointing sunflower strips and so to determine whether cambial growth had been promoted in those strips which were in contact with the pea seedlings. It was hoped that if the plants were examined fairly soon, the brown layer between them would be found still unbroken. Five experiments were made, and they were pickled for examination after from 22 to 24 days. During this period the pea seedlings expanded either five more leaves (in summer), or four more (in autumn). Eleven controls were pickled after the same times, or slightly longer. The sunflower strips were examined in the experiments at about 2 or 3 mm. below the zone of contact, and in the controls at about the same level.

In the eleven controls it was found that there was no secondary xylem nor cambium in the bundles, and no cambium between the bundles. There were no new tangential walls in the pith beneath the cut surface, except in two plants in which these walls were two deep and one deep respectively.

The results of the examination of the five experiments are given in Table I.

TABLE I

Sunflower strips examined at 2–3 mm. below zone of contact

No. of experiment	Cambial layers between bundles	Cambial layers in bundles	Secondary xylem, in layers	No. of new tangential walls in pith, internally to cut surface, in depth	No. of pea leaves expanded during experiment
1	4–6	3–4	2–4	8–10	5
2	2–4	0–2	0	10	5
3	5–7	6–7	4–6	3–4	4
4	0–2 (in 2 gaps not quite complete)	2–3	0	0	4
5	3–5	5–6	4–6	2–3 in one section 9–10 in another	4

From Table I it can readily be seen that the condition of the sunflower strips in the experiments was very different from what it was in the controls. For in all five experiments a cambium had formed both in and between the bundles, and in three of them it had grown considerably. In four of the experiments there had also been several tangential divisions beneath the cut surface of the pith, and these

divisions are also promoted by the cambial stimulus, as the writer has found in *Vicia Faba* (1932 *a*, pp. 90–1). (For they were found to take place strongly in upward-pointing strips of stem, but only very slightly in downward-pointing strips.) It is therefore clear that the cambial stimulus, coming down from the leaves of the peas, had passed across into the strips of sunflower hypocotyl, and travelled down them.

It was next necessary to determine whether the protoplasms of the two plants had united. In experiment No. 4 the two halves separated easily when gently pulled, so that there cannot have been any interlocking of tissues such as regularly takes place before protoplasms unite. But in all the others the halves adhered, and they were therefore examined by sections through the zones of contact, with the following results.

In No. 3, in which the cambial growth was specially strong, the zone of contact was examined by a continuous series of sections from almost the top to the extreme base, and the well-known brown layer of dead material was everywhere found unbroken between the two plants.

In No. 2, three sections were taken at different levels, and the brown layer was found unbroken between the plants in all of them.

In No. 1, several sections were taken, and the brown layer was found unbroken except in one section at one point: here it had snapped, and the tissues of the two plants were in contact for a width of two cells.

In No. 5, the brown layer was found broken at several levels.

Thus in two experiments the brown layer was almost certainly unbroken, and in a third it was probably unbroken. In the remaining two it was probably only towards the end of the period of experiment that it was broken, since breaks of this kind are usually due to renewed growth and division of the cells close to the brown layer, which distort or stretch it. Thus even when the tissues of the pea and sunflower came into contact through breaks in the brown layer, it is unlikely that their protoplasms united in so short a time, even if they are ever able to unite, which is very doubtful. These experiments therefore show that the cambial stimulus can cross a protoplasmic discontinuity, and further that, like the growth regulator of the coleoptile, it can act upon a plant of a widely different family.

Within the zones of contact also the sunflower strips showed distinct cambial growth, though here it was more irregular, probably on account of the pressure. It may also be mentioned that the other

halves of the sunflower hypocotyls, beneath the cotyledons, showed very strong cambial growth both in experiments and controls. This may have been promoted by the green and leafy cotyledons, or by their axillary buds which kept starting to grow. Sections of two experiments and five controls were mounted permanently and may be seen by anyone interested.

In another series of experiments tissues of two plants of the same species, *Vicia Faba*, were pressed together, but pieces of moist linen or muslin, sometimes soaked in gelatine, were placed between them
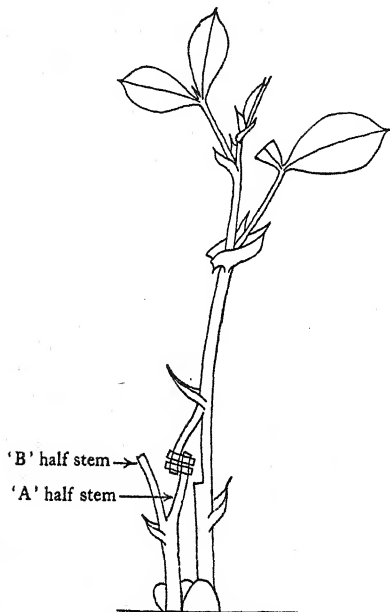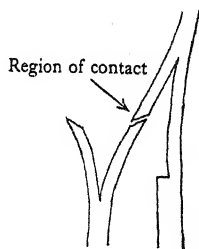
Fig. 2   Fig. 3 (diagrammatic).

to prevent the protoplasms from uniting. The arrangement was the following. Seeds of *Vicia Faba* were sown in pairs close together, and one seedling of every pair was split longitudinally in one of its lower internodes, at a stage when this internode was fully elongated and the next internode but one above it was starting its rapid elongation. One half of the split internode was cut through at the base, so that it formed a downward-pointing strip (see Fig. 2). The other seedling was also split longitudinally in a median plane in one of its lower internodes (the first or second above the epicotyl) at the same stage, and it was then decapitated at the level of the top of the split. All

the cut surfaces were washed, and one of the half internodes of the decapitated plant, which will be called the "A" half, was tied firmly to the downward-pointing strip of the first plant, as is shown in Fig. 2, a piece of moist linen or muslin having first been placed between the two strips. The other half of the decapitated plant, which will be called "B," served as a control. Finally all exposed cut surfaces, and the outer sides of the tied zones, were covered with vaseline.

The strips of the two plants were usually tied with their longitudinal cut surfaces together, in the manner shown in Fig. 2, the length of the tied zones being about 8 mm. But in two experiments, Nos. 1 and 3 of Table II, the strips were cut obliquely at their ends, and tied together in the manner shown in Fig. 3.

After about 4 weeks (in summer), the "A" strips were examined by sections taken a few millimetres below the joined zones, and the "B" strips at the corresponding level. The results of the examination are given in Table II.

TABLE II

Pairs of plants	Separating layer	Manner of contact	Cambium between bundles	Tangential divisions internally to cut surface of pith, in depth	Greatest number of tangential divisions internally to cut surfaces of ring of conducting strands (see text), in depth
1	Linen	Oblique	A. Complete in 3 gaps, not quite complete in 2 gaps	7	6–7
			B. Only just beginning: not complete in any of larger gaps	0	1–2
2	Linen	Sideways	A. None in larger gaps	6–8	6–8
			B. „ „	0	0
3	Muslin	Oblique	A. Complete in all gaps but one	0	15
			B. None	0	1
4	Muslin soaked in gelatine	Sideways	A. Not complete	9–10	8–9 approx.
			B. „ „	0	3–4
5	„	„	A. Not quite complete	7–8	19
			B. „ „	4–5	12
6	„	„	A. None	1–2	7
			B. „	1–2	5

From Table II it can be seen that, although the results were less striking than in the last experiment, nevertheless the "A" halves showed many more tangential divisions internally to the cut surface of the pith and the cut surfaces of the ring of conducting strands than the "B" halves. (When the cut passes through a conducting strand, the new tangential divisions take place in the phloem, the cambium

and the small-celled tissue on the inner side of the xylem.) Also in two of the six plants the "A" halves showed a distinctly stronger development of cambium between the bundles. The bundles themselves were not compared, since it was not certain how far they had already developed at the start of the experiment. The zones that had been tied together separated easily and cleanly from one another and from the linen or muslin between them when lightly pulled. The tissues, therefore, had not grown together and consequently the protoplasms cannot have united. Only in one plant, No. 5 of Table II, did a small piece of tissue remain attached to the muslin on one side.

The divisions beneath the cut surface are promoted by the cambial stimulus, as was shown previously (1932 a, pp. 90–1), since they take place strongly in downward-pointing strips but very little in upward-pointing strips. They are an early stage in a process of regeneration which, in downward-pointing strips, finally restores a complete ring of cambium. It therefore follows that some amount of cambial stimulus must have crossed the protoplasmic discontinuity and the interposed piece of linen or muslin and penetrated into the "A" halves of the decapitated plants. Clearly, however, it was only a small amount of stimulus that entered them, since even in the "A" halves the cambial growth was very much less than in the downward-pointing strips above them.

The experiments here reported show that the cambial stimulus can cross a protoplasmic discontinuity, and incidentally that it can act upon a plant of a widely different family. The simplest interpretation therefore is that it is a hormone, and this interpretation is supported by the fact that, like the growth regulator in the coleoptile, it travels only downwards. But since the cambial stimulus promotes cell division, and not cell-extension like the growth regulator, another possibility must be considered. For it may be suggested that it crosses the protoplasmic discontinuity by means of mitogenetic radiation. But the objection to this is that the cambial stimulus travels comparatively slowly, at a rate which in trees is probably of the order of 3 cm. per hour (see Söding, 1932; Snow, 1932 b, p. 350). It therefore cannot simply *be* mitogenetic radiation. Nor can it be similar to the transmission of a wave of secondary mitogenetic radiation, as discovered by Gurwitsch (1932, p. 279), since this effect is also transmitted very rapidly, in onion roots at about 20 m. per sec. (*ibid.* p. 287). Also it is difficult to see how any kind of transmission which depended on mitogenetic radiation could be limited to one direction only. Never-

theless it is hoped to test by further experiments whether the discontinuity is passed by means of mitogenetic radiation.

Two further points concerning the cambial stimulus may perhaps be mentioned here, though not relevant to the main question of this paper. Firstly the cambial stimulus travels only downwards in the root as well as the stem, in *Vicia Faba* at least. For in that plant if the main root is split longitudinally in a sufficiently old region, from which the secondary roots have grown out, and if one of the halves is cut through at the top so that it forms an upward-pointing strip, then in this half the cambium grows very little, though in the other half it grows strongly. The secondary roots, however, on the upward-pointing strip continue to elongate for some time and may even branch. (In the younger zones of the root it seems that some cambial growth may take place even without any cambial stimulus.)

Secondly, in *Vicia Faba* the cambial stimulus originates not only from growing leaves, but also quite strongly from full-grown leaves. For in decapitated shoots the cambium below the full-grown leaves grows fairly strongly, even if all axillary buds are continually removed as soon as they can be seen. On the other hand in parts of stem above which no leaves are left, the cambium scarcely grows at all.

It was pointed out previously (1932 *b*, p. 350) that the cambia grow strongly in the axes of certain leafless inflorescences and young fruiting branches, and that this made it probable that the inflorescences and growing fruits promote cambial growth. But in order to make certain of this point, it would be necessary to determine whether the cambia in these axes will grow or not if the flowers or young fruits are removed. Münch has recently found by experiment (1932, p. 419) that in two other species (*Pinus strobus* and *Sambucus racemosa*) growing fruits do *not* promote cambial growth. Certain problems concerning the polar movements of the cambial stimulus and the growth regulator, when compared and contrasted in stem, leaf and coleoptile, were also pointed out previously (1932 *b*, pp. 350–1).

SUMMARY

The cambial stimulus can pass across a protoplasmic discontinuity and through an interposed piece of moist linen or muslin. It can also act on a plant of a widely different family. These results, together with other facts known previously, indicate that it is probably a hormone.

20-2

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THE INFLUENCE OF X-RADIATION ON *ATRIPLEX HORTENSIS* L.

By EDNA LOUISE JOHNSON

(With 2 figures in the text)

ORACH, or French spinach (*Atriplex hortensis* L.) is an amaranthus-like plant which is cultivated in European countries where it is used much like spinach. It is an annual, grows about 4 ft. in height and has arrow-shaped leaves of soft texture. The flowers are less conspicuous than the fruit which is covered by persistent bracts. There are three main types of orach, based on the colour of the leaves. The white variety, with pale, almost yellowish leaves, is most commonly grown for food. The red variety, which is occasionally cultivated as an ornamental plant, has foliage of dark red colour which disappears in cooking. The green variety is the most vigorous type. Seeds of the white and green varieties could not be obtained in America but were secured through Sutton and Sons of Reading, England.

After preliminary studies with the red variety had indicated that it grew readily in the greenhouse, could be easily transplanted, matured rather quickly, and was relatively sensitive to X-radiation, a morphological and physiological study of the three varieties of *Atriplex* was undertaken to determine whether the difference in their responses to an environmental factor such as radiation was due to physiological peculiarities of the plants themselves. Physicians have long recognised that members of the human species differ greatly in their reaction to X-rays, blondes being more sensitive than brunettes. Are there, then, blondes and brunettes among plants? Would the presence of red sap in the *Atriplex* screen the protoplasm from the injurious effects of the rays?

When results indicated that there was a difference in the radio-sensitivity of the three varieties, it seemed desirable to know whether those plants showing the greatest retardation of growth and highest percentage of anomalies as the result of treatment would show also the greatest decrease in catalase activity.

EXPERIMENTAL METHODS AND RESULTS

(1) *Growth of X-radiated seedlings of red* Atriplex

Two groups of seedlings of the red variety of *Atriplex* from 1 to 2 weeks old were irradiated with one dose of X-rays. Within 2 weeks both the experimental and control seedlings were transplanted so that there were but two in each pot. At the time of transplanting, the experimental seedlings were usually about one-half the size of the controls, and the leaves of the red variety were rather brownish in colour, and were pitted and pebbly in appearance.

The leaf deformities and stem lesions, which became evident soon after irradiation, persisted. This was shown by a count of abnormal leaves in Group II made 65 days after irradiation. Each irradiated plant showed an average number of 4·6 abnormal leaves, while the controls showed none. Stem lesions, longitudinal splits in the lower region of the stem, were present in 52·4 per cent. of all plants which had been irradiated. None were seen in the controls.

These earlier experiments as well as later ones demonstrate decreased height of the red irradiated plants as compared with the controls. In one series for instance, as shown in Table I, 9 weeks after irradiation, the percentage decrease was 38, while at maturity, it was 10.

TABLE I

Growth of stem of the red variety of Atriplex hortensis

(Set-up used or radiation of plants: 52 kV., 5 ma. current, 20 min., distance 30 cm.)

Group I (15-day seedlings treated)

	Control	Irradiated	Percentage difference in treated plants*
No. of plants	10	10	
Av. stem height (in cm.):			
62 days after treatment	42·1	25·9	−38·5
76 ,, ,,	70·4	56·2	−20·2
111 ,, ,,	80·1	71·9	−10·2

Group II (8-day seedlings treated)

	Control	Irradiated	Percentage difference in treated plants*
No. of plants	29	63	
Av. stem height (in cm.):			
65 days after treatment	27·5	20·7	−24·7
81 ,, ,,	50·2	43·2	−13·9
115 ,, ,,	66·3	62·8	−05·3

* The minus sign indicates that the treated plants exhibited less growth than the controls.

In a second series, reduction in height dropped from 25 per cent. at first measurement to 5 per cent. at maturity. These figures show that in *Atriplex*, as in most other plants studied (3, 4, 5) there is a general tendency for plants as they grow older to show recovery from the retarding influence on growth which accompanies X-radiation.

(2) *Comparison of internodal length in treated and control red* Atriplex *plants*

Measurements of the length of each internode were taken at maturity in two separate groups of the red plants in order to determine

TABLE II

Internodal lengths of stems of the red variety of Atriplex hortensis

Group I

	Control		Irradiated		
Internode No.	Plants measured	Av. internodal length (cm.)	Plants measured	Av. internodal length (cm.)	Percentage difference in treated plants*
1	10	3·2	9	3·6	†
2	10	1·6	9	1·1	−31·2
3	10	1·9	9	1·3	−31·6
4	10	2·1	9	1·5	−28·5
5	10	3·4	9	1·7	−50·0
6	10	4·2	9	2·3	−45·2
7	10	5·4	8	3·7	−31·5
8	10	6·8	8	5·3	−22·0
9	10	8·2	8	6·4	−21·9
10	10	7·5	8	7·5	0·0
11	10	5·7	8	4·1	−28·0
12	10	5·5	8	3·9	−29·0

Group II

	Control		Irradiated		
Internode No.	Plants measured	Av. internodal length (cm.)	Plants measured	Av. internodal length (cm.)	Percentage difference in treated plants*
1	29	4·9	63	6·5	†
2	29	0·8	63	0·5	−37·5
3	29	1·7	63	1·5	−11·8
4	29	2·3	63	1·7	−26·1
5	29	3·2	63	2·6	−18·7
6	29	4·6	63	3·7	−19·6
7	29	6·8	62	5·4	−20·6
8	29	8·3	62	6·8	−18·1
9	29	9·0	62	7·9	−12·2
10	27	9·1	61	7·8	−14·3
11	22	8·3	56	8·3	0·0
12	18	10·0	39	7·2	−28·0

* The minus sign indicates that the treated plants exhibited less growth than the controls.

† Hypocotyl region which had developed before irradiation.

whether the decreased growth was due to shortened internodes or to a decreased number. Measurements given in Table II are for only the lower twelve internodes, as in the region of the inflorescence there was difficulty in distinguishing clearly the individual nodes. In one group containing nineteen plants, the percentage difference in average internodal length varied from a decrease in the treated plants of 50 per cent. in the fifth internode to none in the tenth. All the internodes formed after treatment, however, with one exception, showed a decrease in length of more than 20 per cent. In the second group where internodes of ninety-two plants were measured, the greatest percentage decrease in internodal length of treated plants was 37 per cent.; this occurred in the first internode above the hypocotyl. A very considerable decrease was evident in all internodes, except in the eleventh where the average lengths were the same.

(3) *Radiosensitivity of three coloured varieties of* Atriplex hortensis

A comparative study of the effects of radiation upon the three different coloured varieties of *Atriplex* was made to determine whether they would respond similarly to radiation. Ten-day seedlings of the three varieties were given the same amount of radiation (52 kV., $7\frac{1}{2}$ ma., 20 cm. distance, for 23 min.). Two weeks afterward, the new plants showed types of anomalies which are common to many other irradiated plants. The leaves were smaller, narrower, misshapen and much more mealy in appearance than the controls. The leaves of the treated white plants seemed more elongated than the controls, whereas the leaves of the treated red and green plants were smaller and more rounded than the check plants. Abnormally shaped leaves of the treated red plants as well as their irregular placing are shown in Fig. 1. Often two or more petioles arise from the same side of the stem. Stem lesions, dichotomous branching and increased lateral branching are other irregularities typical of treated plants. Results given in Table III indicate that the 46-day seedlings showed leaf deformities and altered phyllotaxy in 58·3 per cent. of the treated red seedlings, in 60 per cent. of the white, and 80·8 per cent. of the green.

Many of these anomalies were still evident at maturity. The white showed the highest percentage of plants with stem lesions and dichotomous branching; the green variety had the greatest number of plants with flattened stems, while lateral branching occurred in as many as 32 per cent. of the treated red plants.

Fig. 1. Control and irradiated (right) plants of the red variety of *Atriplex hortensis* 78 days after irradiation with 2500 *r* units. Note the reduced root development and lessened height of the treated plant. Altered phyllotaxy is evident in the experimental plant and leaf deformities are shown clearly at the tip and right side.

TABLE III

Growth and development of three varieties of Atriplex
following X-radiation

	Red		White		Green	
	Control	Irrad.	Control	Irrad.	Control	Irrad.
	46 days after planting					
No. of plants	45	36	13	15	19	26
Av. height (in cm.)	9·2	6·1	4·0	3·5	9·0	6·0
Percentage decrease in height of irradiation		33·7		12·5		33·3
Percentage of plants showing leaf anomalies and altered phyllotaxy		58·3		60·0		80·8
	156 days after planting					
No. of plants	45	28	11	12	19	21
Av. height (in cm.)	53·6	40·3	62·2	50·5	56·8	50·0
Percentage decrease in height of irradiation		24·8		18·8		12·0
Percentage of plants showing anomalies						
Stem lesions		2·4		16·6		
Flattened stem		7·1		25·0		38·1
Dichotomous branching		14·3		33·3		14·3
Lateral branching		32·1		25·0		19·0
Age at time of blossoming (days)	78	90	85	93	79	83
Percentage delay in blossoming of treated plants		15·4		9·4		5·0

Measurements of height of controls and treated 46-day seedlings indicated that the percentage decrease in growth of treated plants as compared with the controls was similar in the three varieties. Measurements of the younger plants showed 33·7 per cent. decrease in stem height of treated red orach seedlings, 12·5 per cent. in the white, and 33·3 per cent. in the green. More variation in results was noted when measurements of the mature plants were taken. The percentage decrease in the total height of the red experimental plants was 24·8, in the white 18·8, and in the green 12.

Blossoming was retarded in experimental plants. The controls of the red and green varieties blossomed sooner than did the white. There was a greater difference between time of blossoming of control and irradiated in the red than with the other varieties. In the former,

the percentage of retardation in blossoming was 15, while that of the white was 9, the green 5.

It appears that the red and white varieties show the greatest amount of injury while the green shows the least, if one considers percentages of decreased growth, irregularities of development, and delayed blossoming which accompanies irradiation.
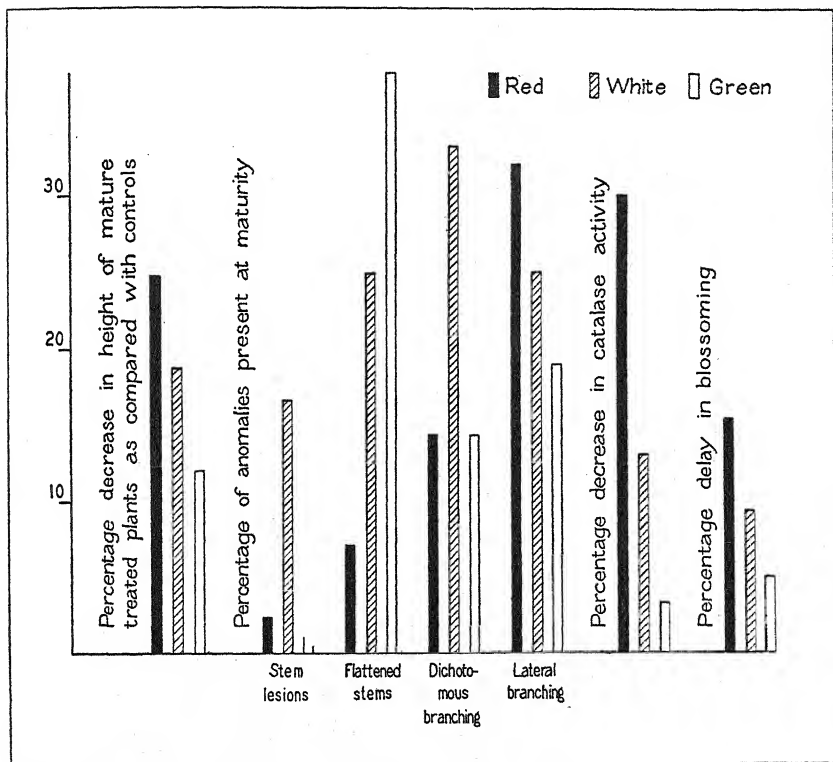
Fig. 2. Comparative effects of X-radiation upon three coloured varieties of *Atriplex*. Percentage differences between treated and control are indicated by rectangles.

(4) *Comparison of catalase activity in treated and control seedlings of three varieties of* Atriplex

Experiments carried on to determine whether inhibition of growth and production of anomalies which occur in treated seedlings were associated with decreased catalase activity indicate that the red and white varieties which show the greatest amount of injury also show

the greatest reduction in catalase activity and most delay in blossoming. These facts are graphically illustrated in Fig. 2, where it is indicated that the red shows the greatest percentage of difference between control and irradiated plants in four of the conditions considered, while the green exhibits the least difference in four cases.

For the catalase experiments, an apparatus modified from that of Appleman [1] was used. The water bath was kept between 19 and

TABLE IV

Catalase activity of 50-day Atriplex hortensis *seedlings irradiated with 2500 r units*

Green variety (24 days after irradiation)

Control

Weight of green material (gm.)	Height of plant (cm.)	Cc. O_2 liberated in min.			O_2 per gm. weight (cal.)
		2	5	10	
0·2786	5·5	13·5	25·0	31·8	114·1
0·3374	5·5	21·5	34·2	38·0	112·6
0·3330	6·5	18·6	32·7	36·1	108·5
					Av. 111·7

Irradiated

0·2641	4·5	16·2	27·7	33·1	125·4
0·1578	4·7	6·8	11·8	14·5	91·9
0·1347	5·2	6·3	11·2	14·4	106·7
					Av. 108·0

White variety (26 days after irradiation)

Control

0·1569	—	7·2	13·6	13·8	87·9
0·1382	2·3	6·9	11·7	13·3	96·4
0·2397	5·0	9·4	18·8	22·9	95·5
					Av. 93·2

Irradiated

0·1509	4·0	6·4	9·6	11·0	72·9
0·1222	4·0	6·1	9·7	11·6	94·9
0·0562	3·3	2·4	3·5	4·2	75·3
					Av. 81·0

Red variety (40 days after irradiation)

Control

0·2340	Tip of plant used in determination	11·6	20·1	24·0	102·7
0·1739		9·5	15·7	18·7	107·6
0·1213		4·0	7·4	9·0	74·8
					Av. 95·0

Irradiated

0·1149	Tip of plant used in determination	3·2	5·2	5·9	52·1
0·1435		5·7	9·2	10·4	72·5
0·0841		3·4	5·4	6·3	74·7
					Av. 66·4

Summary of results shown in percentages.
Decrease in O_2 per gm. green weight (calculated) of 50-day treated seedlings: green 3·3; white 13·1; red 30·1.

TABLE V

*Catalase activity of 50-day seedlings irradiated
with 2500 r units*

Variety	Average height (in cm.)	Percentage decrease in catalase as compared with controls
Red	Only tip used	30·1
White	3·6	13·1
Green	5·8	3·3

21° C. All results were calculated to standard conditions of temperature and pressure. Green tissues were ground in a mortar with an excess of calcium carbonate which kept the material alkaline and then washed quantitatively into the shaking bottle with distilled water. After the addition of hydrogen peroxide the whole was shaken evenly for 10 min. by means of an automatic shaker. Determinations were run in triplicate, and from the averages obtained the average amount of O_2 per gm. of green weight was then calculated.

Results given in Tables IV and V show that the treated seedlings in each of the three varieties showed decreased catalase activity. In terms of percentages, the treated red seedlings gave 30·1 per cent. decrease, the white 13·1 per cent., while the green showed a decrease of only 3·3 per cent.

DISCUSSION

Atriplex hortensis L. was found to be relatively sensitive to X-radiation as was indicated by the deformed, pitted, and puckered leaves of the new growth. Decreased growth of the treated seedlings was more evident during the early stages, but even at maturity there was still a decrease in height. *Atriplex* thus agrees with other plants [3,5] in showing that when given one dose of X-rays in the seedling stage, their growth is inhibited for a considerable period, after which there is a tendency for the plants to recover from the inhibiting effects of radiation and in some cases almost to equal the controls in height.

The decrease in height of irradiated plants was due to a shortening of the internodes rather than to a reduction in their number. The greatest decrease in length occurred in the first internode above the hypocotyl. The seedlings were irradiated when the cotyledons were fully expanded before new foliage leaves had appeared. The meristem is strongly affected by the rays, and growth is thus inhibited more in this internode than in those which develop later.

All three coloured varieties of *Atriplex* agree in showing leaf abnormalities and altered phyllotaxy. The treated white variety, however, showed leaves which were much more ligulate than the controls, while the treated red and green plants produced leaves which were smaller and more rounded than the check plants. Anomalies occurring in the irradiated *Atriplex* plants are of the same nature as those found in treated plants of other species—stem lesions, flattened stems, dichotomous branching of the main central stalk, and a tendency toward lateral branching.

Each of the three varieties showed considerable reduction in total height of treated plants at maturity and some retardation in blossoming. Considering all responses which indicate influence of X-ray treatment, the red and white varieties manifested greatest injury while the green showed the least.

It has been demonstrated in some cases that catalase parallels the general metabolic activity of the organism. Heinicke [2] states that it is very probable that the activity of catalase is a more sensitive measure of the metabolic status of tissues than the usual chemical analysis and that it may serve as an indication of the physiological responses of plants to various cultural conditions or treatments. Measurements of catalase activity of the treated and control red, green and white seedlings gave results which seem to agree with Heinicke's conclusions, for the treated red and white varieties showed the greatest decrease in catalase and the green, which had shown the influence of irradiation the least, gave the slightest difference in catalase determination for the treated and check plants.

SUMMARY

A comparative study of three coloured varieties of *Atriplex hortensis* indicates that an environmental factor such as X-radiation may cause a differential growth rate in the three varieties. The red form showed the greatest percentage decrease in height of treated plants as compared with the controls both during its earlier stages and at maturity. The lessened height is caused by shortening of internodal distance rather than by decrease in number of internodes.

The irradiated white plants gave the highest percentage with stem lesions and dichotomous branching; the green variety exhibited the greatest number of plants with leaf abnormalities, altered phyllotaxy, and flattened stems, while lateral branching was most frequent in the red variety.

Catalase activity, often used as an index of vitality and vigour of plants, was most reduced in the red irradiated seedlings, followed in turn by the white and green varieties, thus seeming to parallel reduced growth and delay in blossoming.

Experiments have indicated that certain plant families are more sensitive to irradiation than others; species in the same family differ in their responses to the same dose. This present study indicates that varieties in the same species also differ in their physiological and morphological response to radiation.

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A NOTE ON THE UTILISATION OF AMMONIUM AND NITRATE BY HIGHER PLANTS

By JOSÉ H. PARDO, M.A., M.Sc., Ph.D.

THE direct absorption of ammonium by higher plants and its effect on their development, as compared to that produced by the absorption of nitrates, is a problem of plant nutrition which has been the object of considerable research in recent times.

The usual procedure in investigations of this kind, is to carry out culture experiments of which one section consists of plants receiving ammonium salts, while in another section nitrogen is supplied as nitrate salts, conditions being otherwise as uniform as possible for the two lots of plants. Once the culture plants have reached a favourable stage of development, they are harvested and the two lots are compared to determine which nitrogen form has produced the better growth under conditions that prevailed during their culture growth, both as regards climate and the particular nutrient medium employed. For this purpose the determinations of green weights or dry weights (or both) are used.

The average total plant weight results have thus been taken as the most important criteria for estimating the comparative value of NH_4 and NO_3 as nitrogen sources for various species of plants. In fact, little attention has been paid to other aspects of the question, particularly regarding nitrogen absorption correlated with growth response. Therefore, an attempt is made, in this short paper, to demonstrate the relative values of what is here called "the efficiency" of ammoniacal nitrogen as compared to that of nitrate nitrogen.

By "efficiency" is meant, the weight of plant material obtained per unit of the nitrogen absorbed as one form or the other; absorption being estimated by analysis of the dried plant, following the ordinary Kjeldahl method for total nitrogen as NH_4 (not including nitrates). It may, thus, be represented by the ratio:

$$\frac{\text{total dry weight of plant material}}{\text{weight of nitrogen in plant material}}.$$

The following figures, taken from some of the writer's results of solution-cultures experiments with sugar-cane, may illustrate the point.[1]

Test No.	Nitrogen source	Aver. total dry weight (gm.)	"Total nitrogen" in dry matter		"Efficiency"
			%	gm.	
I	$Ca(NO_3)_2$	117·00	0·923	1·080	108·3
	$(NH_4)_2SO_4$	68·90	2·167	1·493	46·1
II	$Ca(NO_3)_2$	7·36	2·067	0·152	48·4
	$(NH_4)_2SO_4$	8·96	2·501	0·225	39·8
III	$Ca(NO_3)_2$	8·90	1·742	0·155	57·4
	$(NH_4)_2SO_4$	8·98	2·244	0·201	44·7

First of all, the average total dry weight figures show that, whereas in test I, nitrate as $Ca(NO_3)_2$ led to decidedly better growth of sugar-cane than ammonium as $(NH_4)_2SO_4$, the latter proved to be slightly superior in this respect to the nitrate in test II, while both forms were equally effective in the development of the plants of test III.

It must be mentioned that these three tests were carried out under different environmental conditions, not only with regard to climate—in spite of the fact that the experiments were carried out in greenhouses—but also with regard to the nature of the nutrient solutions used.

These apparently contradictory results demonstrate, therefore, the highly complex nature of the problem, and the considerable influence which other factors of the medium of growth may exert at times on the comparative value of one nitrogen form and the other for the development of a plant. These factors need not be here considered, but it can reasonably be expected that their influence should be the strongest in culture experiments with plants that do not appear to have any natural preference for ammonium or nitrate, and consequently may do equally well on both forms. In fact, this seems to be the case with the development of sugar-cane as a whole.

The figures for total nitrogen as a percentage of the dry matter show, on the other hand, that plants fed on ammoniacal nitrogen were always of a higher nitrogen content than similar plants treated with nitrate nitrogen. This is best shown by the nitrogen figures of plants of test I, indeed to such an extent that, although ammonium plants weighed only about two-thirds as much as the nitrate plants, the weight of their nitrogen content was almost 40 per cent. greater than

[1] The solution cultures were made with cuttings detached from the parent plant before the experiment was started. An aeration method was employed and the nutrient solution was renewed every 3 days, or twice a week. The variety of cane used was POJ 2878.

that of the latter. Thus, regardless of its stage of development, the sugar-cane plant shows a greater absorption of nitrogen in the form of ammonium than in the form of nitrate (case I *versus* case II or III).

As noted before, these total nitrogen determinations do not take into account any nitrates that might be present as such in the plant tissues; but nitrate accumulation in the sugar-cane plant does not appear to amount to any considerable value, and certainly not to such an extent as to account for the inferiority shown by nitrate plants in total nitrogen content. Nevertheless, the results of nitrogen analyses and the calculations derived from them, should be considered as being approximate figures only.

The "efficiency" figures demonstrate, in all three tests, that a greater plant weight was produced per gram of nitrogen when this was absorbed as nitrate than when in the form of ammonium. On the other hand, the comparative value of nitrate and ammonium as nitrogen sources for sugar-cane, if judged merely by the total plant weight results of these tests, would lead us to the conclusion that these nitrogen forms appear to be "equally good" for that plant.

Optimum development must, undoubtedly, be kept as the first consideration in estimating the comparative values of ammonium and nitrates, as nitrogen nutrients for a plant; but it is also apparent that their "efficiency" values, *i.e.* the dry weight, produced per unit weight of nitrogen absorbed, should also be taken into consideration, since the values may, as shown above, be so strikingly different.

AN ANTARCTIC POLLEN-GRAIN; FACT OR FANCY?

By A. C. SEWARD

(With 1 figure in the text)

IN 1914 I described under the name *Pityosporites antarcticus*[1] a small body found in the siliceous matrix of a partially decayed stem discovered by Mr R. E. Priestley in a boulder on the Priestley Glacier on the Antarctic Continent. The stem was originally called *Antarcticoxylon Priestleyi*: subsequently Professor Walton, after investigating *Rhexoxylon* stems from South Africa, recognised Priestley's specimen as a species of that genus and renamed it *Rhexoxylon Priestleyi*[2]. Two "small bodies" were mentioned in my description and one of them was photographically reproduced. I wrote[3]: "the resemblance of the one shown in the figure to a winged microspore of *Pinus* and other Abietineae is too close to be explained by any accidental similarity to a crushed parenchymatous cell....The central part of the spore is bounded by a comparatively thick wall, and on each side a portion of the membrane is expanded into a bladder characterised by a fine surface reticulum as in recent pollen-grains." In my recent examination of the section containing the specimen of *Pityosporites* figured in 1914, and now refigured, I did not find the second example mentioned in the original description. Reference was made to other records of Mesozoic spores comparable with that from the Beacon Sandstone of Antarctica and it was pointed out that, while *Pityosporites antarcticus* agrees most closely with Abietineous pollen, on geographical grounds it would seem more likely to be Podocarpineous.

In 1925 Professor Walton[4], in a paper on various types of fossil wood from South Africa, stated that some imperfectly preserved stone-cells in a stem of *Rhexoxylon Priestleyi*, from a South African locality, reminded him of *Pityosporites antarcticus*. He wrote: "I am inclined to believe that *Pityosporites antarcticus* is a crushed or irregular-shaped stone-cell in the state of preservation common in this

[1] Seward (1914), p. 23, Pl. VIII, fig. 45.
[2] Walton (1923).
[3] Seward, *loc. cit.*
[4] Walton (1925), p. 11.

(*Rhexoxylon*) South African specimen, in which the pitted nature of the thick wall is represented by a reticulum in the carbonaceous remains of the wall." He added: " the two-lobed shape of *Pityosporites antarcticus* has not been seen in the new specimen, but many structures which might have been interpreted as simple reticulately ornamented microspores are present, were it not that their relation to definite stone-cells is revealed unmistakably." Unfortunately Professor Walton did not publish any drawings or photographs of the thick-walled cells which he thought the unwary might describe as pollen.
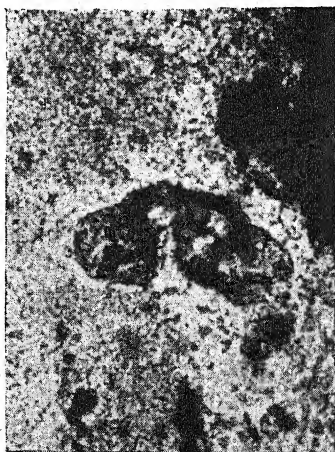
Fig. 1. *Pityosporites antarcticus* Sew. ×400.
(Photograph by Dr Hamshaw Thomas.)

In 1928 Mr Edwards[1], writing on the occurrence of *Glossopteris* in the Beacon Sandstone of South Victoria Land, intercalated an *obiter dictum* on my unfortunate pollen-grain. "I agree," he wrote, "with Professor Walton in regarding the supposed spore, *Pityosporites antarcticus*, as merely a shrivelled pith-cell."

The question whether or not my Antarctic spore is just fancy, or possibly an instance of "Fancy with fact is just one fact the more," is of more than personal interest because of the discovery in recent years of winged spores of *Glossopteris*, a genus recorded from two localities in Antarctica. Dr Hamshaw Thomas, who is at present engaged in preparing an account of *Glossopteris* spores from South African beds, kindly took the photograph of *Pityosporites antarcticus*

[1] Edwards (1928), p. 327.

which is here reproduced: it shows the characters rather more clearly than the photograph published in 1914. The dimensions, as previously stated, agree generally both with those of recent coniferous pollen and the spores of *Glossopteris*. On focusing through the thickness of the "small body" one obtains the impression that in the preparation of the section part of the body may have been cut away. If this small body is a shrivelled pith-cell it is a most remarkable example of Nature's cleverness in setting up false sign-posts. The occurrence of an Abietineous or a Podocarpineous pollen-grain in an Antarctic stem, even though the stem may have travelled far before it sank into the sand of the boulder, raises a difficult problem which the shrivelled-cell hypothesis would readily solve: the discovery of winged pollen of *Glossopteris* and of other extinct genera, *e.g.* of the Caytoniales, supplies another possible solution. Whatever may have been the parent plant, I venture to think that *Pityosporites antarcticus* should be granted a new lease of life and not added to the long list of records that are valueless save as giving point to the warning that things are not what they seem.

Dr Godwin and Mrs Godwin, who are engaged in pollen-analysis of peat of various ages, made a careful examination of the Antarctic specimen: they, with Dr Hamshaw Thomas and myself, are favourably disposed towards the pollen-grain interpretation. Dr Godwin points out that the bladders seem to be more voluminous than in *Pinus* and embrace a large proportion of the whole grain.

My own view is that the original description is probably correct. One may fairly ask for more convincing evidence to the contrary than has so far been presented before discarding *Pityosporites antarcticus* as a *nomen nudum*.

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REVIEWS

Gastromycetae. By ED. FISCHER. (*Die Natürlichen Pflanzen-familien*, von A. ENGLER und K. PRANTL, zweite Auflage herausgegeben von A. Engler und H. Harms, Bd. 7 *a*). Leipzig: Wilhelm Engelmann, 1933. Pp. iv + 122, 91 figures. Unbound, 20 RM.; bound in half-leather, 26 RM.

In revising his monograph on the Gastromycetes, after an interval of more than thirty years, Professor Fischer has presented us with one of the best systematic accounts of the higher fungi. This comparatively small group has been taken from the rest of the Basidiomycetes and in this standard series on systematic botany conceded a special volume for its sole description. It seems a trifle lavish to have published such a slip separately, but then the author is entitled to a distinguished place in mycology, and the book is a fair compliment both to himself and the subject of which he is the master. Of all fungi, the Gastromycetes deserve this rank. They command, it is true, no general interest, but they have the strangest and most varied fruit-bodies, behind which must lie an enormous evolutionary history, teeming with incident, at present, unrealised; they are, in fact, almost unbelievable, coming rather as fancies from dreamland than the dire necessities of a savage earth; they far excel all other filamentous Xerophyton in complexity and perfection. An hour among these pages, if it does not numb the imagination, will disclose the most astonishing problems of fungous organisation.

The plan of the book is that of the original. The text has been thoroughly revised and extended from 71 to 119 pages. All essential advances in knowledge which have occurred during the last three decades have been incorporated and the bibliography is complete. Errors and misprints are remarkably few: we note only "british" (p. 46) where a capital is preferable, and "pötzlich" (p. 51, line 21 from the bottom), which however is the more expressive. The printing is excellent —captions, citations, names and keys being admirably clear and botanically correct. The number of figures has been increased and most are as well produced as in the original; only some of the additions, e.g. Figs. 15, 23, resemble the comic-cuts which mar the companion volume. Sachs' drawings of *Crucibulum vulgare*, perhaps the most suggestive and inspiring which have yet been made for this group of fungi, have profited by the re-arrangement. Particularly valuable improvements are the exact references to generic names, giving both the date and derivation, the citation of type-species wherever possible (thus denoting the generic names), the citation of special references under each genus as well as the principal literature under the family headings, and the accurate specific data which enable one to diagnose the main species in most genera. With this work, alone, one should be sufficiently informed to contribute to the study of the group, and especially is it welcome to mycologists in the tropics where libraries are the least complete and opportunities the greatest.

As for the systematic treatment, Professor Fischer has also followed the lines of the original. The Melanogastraceae, Hydnangiaceae, Geastreae and Glischrodermataceae are new families, the first being removed from the Sclerodermatineae to the Hymenogastrineae and the last following Carleton Rea's classification. The number of valid genera has increased from 67 to 105, mainly as a result of the exploration of the tropics and the southern hemisphere during the last twenty years at Lloyd's instigation. No new genera are proposed. We may reckon about 600 species. It is not quite so easy to follow the specific descriptions, as the keys have been compressed into continuous paragraphs, but this arrangement has doubtless saved much space and the information is on the whole accurate and sufficient. We do not think that the author has been entirely happy in some of his subdivisions. Thus the pectinate Geasters cannot be so sharply delimited from the fimbriate, e.g. *G. Dybowskii* and *G. McOwani* are much closer to the fimbriate *G. velutinus* and *G. fornicatus* respectively than to

the other Pectinata with which they must be classed. But these are minor points of temporary convenience, and the author himself admits that many changes will be wrought when the group has been more thoroughly investigated, and, we would add, particularly in the microscopical aspect. As it is, the classification is so convenient that with a very little effort one may obtain a conspectus of the whole variety of species and genera at any level of organisation.

Professor Fischer has also provided, so far as possible, an introduction to the morphology. He has followed Lohwag's interpretation of the fruit-body along the empirical lines of descriptive anatomy and recognised five types according to the arrangement of the tramal plates and hymenium. For details one may refer to original papers and especially to the comparative accounts of Gäumann, Dodge and Lohwag, though we confess we have always wondered at the latter for ascribing the group to the Protista, and we cannot find in the whole work a genus which strictly corresponds with the multaxial "multipileate type," unless it be Broomeia or Diplocystis: the Clathraceae are surely monopodial with a single, alveolate or reticulate pileus, cf. Morchella.

Despite the excellence of the work, we feel however that there are several omissions. We regret the lack of biological perspective. The introduction might well have been extended, because it is not yet possible to incorporate the group scientifically in general botany, and where else should one look for enlightenment? Though the discovery of its true nature rests with the future, the author might have directed us in many attractive ways. The field of investigation is immense: even the spores, for example, in many genera have never been seen to germinate, e.g. the Phalloids. Why is the form of the basidium so varied? Have the basidia no power whatever of spore-discharge? How are the spores set free and why have they such an elaborate structure, cf. the Tuberales? Diagrams of the types of basidia and spores characteristic of the main genera or families would have brought such points vividly to mind. Similarly diagrams of the construction of the fruit-body in typical genera would have enabled one to have appreciated their relationship so much more readily. Then, perhaps, might one have understood why there are so many perfect forms, so few imperfect, cf. · Geaster-Trichaster, Lycoperdon-Calvatia, Cyathus-Nidularia, Aseroe-Lysurus, Phallus-Itajahya; and the homoplastic forms could have been contrasted, e.g. Astraeus-Geaster, Scleroderma-Calvatia, Pseudocolus-Mutinus, Simblum-Staheliomyces, etc. We look vainly for assistance. And why is there such variety and how do they all work? One must search each genus for information and sort it out oneself. Those marvels of hyphal engineering— Cyathus, Sphaerobolus, Clathrus and Dictyophora—are hidden away in systematic pigeon-holes. We find no mention that the peridioles of Sphaerobolus may shoot 14 feet into the air, that the gaudy phalloids stink and are dispersed by flies, that the mephitic Hymenogastrineae lure the wild pigs, deer and rodents which grub them up, eat and disperse them (and so, perhaps, their elaborate epispore), that earth-stars smoke, Bovistas roll across the fields, or that Podaxis may be perennial, of the troops of Geaster, the hordes of *Lycoperdon pyriforme*, the rings of Calvatia, that acre of Lysurus, or the seven billion spores of the giant puff-ball. They might be so many lifeless toys. And we miss too the historical element, which humanises the subject. Can Lloyd's Notes and Puff-ball Letters, and Petch's labours in Ceylon, pass unremarked? Made Vittadini's dog and Tulasne's pig no contribution to the science?

Professor Fischer upholds the Brefeldian view, considering that the most complicated Gastromycetes, as the Phalloids and the Podaxineae, have evolved from the subterranean Hymenogastrineae, parallel with the Agaricaceae and Polyporaceae, and like them are ultimately to be derived from a resupinate thelephoraceous ancestor: he suggests, following Lohwag, that they may even have given rise to the Agaricaceae and Boletaceae. Is there not the alternative that the Gastromycetes are degenerate agarics, merely toadstools that have gone wrong, with a disorganised gill-system and failing expansion-mechanism? Those astonishing genera, Elasmomyces and Secotium, surely give the key to the problem and invert the author's hypotheses. Take the figures of Podaxis,

Elasmomyces, Secotium, Chamonixia, Arcangeliella, Hysterangium and Hymenogaster and see the juvenescence of the agaric fruit-body! The stem or columella is the axis and primordial shaft, the outer peridium the universal veil, the inner peridium the pileus and limb, the gleba the disorganised lamellae. The Hymenogastrineae are then but unexpanded primordia, which generally are subterranean in the advanced, humicolous agarics, e.g. Amanita, Cortinarius or Psalliota. We may expect them to be polyphyletic on many toadstool lines and hence the significance of the characters of spore and basidium. Many families are clearly artificial groups of homoplastic forms arrived at the same degree of decadence, though one cannot doubt that the genera with their exceedingly specialised features are natural. And at any stage some wayward point of construction may have been worked out and a new family born with its own peculiar degenerates, e.g. the Phalloids and Nidulariineae. We would invert Professor Fischer's series—Hysterangium, Phallogaster, Protubera, Clathrus— for we see no such prospect before a ball of hyphae working underground and on the principle of least surface. We are at the cross-roads again and Professor Fischer has taken one turning. Which is right can be decided only when the Gastromycetes have been worked out in terms of hyphae and after the simpler Basidiomycetes are thoroughly understood: such will be labour of many years and a task for the youngest eyes. But, of whatever little faults one may accuse it, this monograph must remain the standard of reference for fullness and accuracy for at least another generation. And in bringing together all the scattered writings on these organisms, and in consummating his life's work of nearly fifty years, the author has led us to the brink of discovery and given us a parting handbook, which is a model of systematy.   E. J. H. C.

Phytopathological and Botanical Research Methods. By THOMAS E. RAWLINS. London: Chapman and Hall, Ltd.; New York: John Wiley and Sons. 1933. Pp. ix + 156. Price 15s. 6d. net.

This volume is apparently intended to assist the advanced student who is about to undertake a piece of research work. It is divided into three sections. Part I (3 pages) stresses the need of a thorough review of the literature on the subject chosen, and includes a useful list of publications from which literature can readily be traced, though the omission of Lindau and Sydow's *Thesaurus* is somewhat surprising. Part II (96 pages) comprises four chapters dealing respectively with microscopic methods, culture methods, methods used in virus studies, and a few miscellaneous experimental methods. In the first of these (Chapter II), in addition to a description of the methods of fixing and staining found to be most satisfactory in phytopathological work, there is a short account of the theory of staining, and a description of the methods used in the identification of crystals and of non-crystalline plant constituents, as well as an outline of the principles of micrometry. In Part III (5 pages) simple methods in use for the statistical interpretation of results are briefly described. No attempt is made merely to enumerate experimental methods in common use in the laboratory but "considerable work was done to develop new experimental methods and to improve technics described by other workers. The methods described are those which were found to be most satisfactory." A valuable feature of the book is a list of 960 references to original papers, classified under Histology and Cytology, Microchemistry, Culture Methods, Viruses, Miscellaneous Methods and Interpretation of Experimental Results. There is also an adequate index. A few misleading statements have been noted; on p. 81, for instance, the nutrient medium referred to as *Horne and Mitter's Medium* was prepared and first used by W. Brown, and is usually known in England as *Brown's Medium*. The subject matter is very sketchy and unbalanced, and, in places, notably in the chapter dealing with methods used in virus studies, quite inadequate. As a handy reference hook, however, it should prove helpful to plant pathologists.
                                                                    W. C. M.

SAP PRESSURE AND THE MOVEMENTS OF SAP

By W. O. JAMES and H. BAKER

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(With 9 figures in the text)

INTRODUCTION

THE exudation of saps by roots and the lower regions of stems has been an object of scientific study since the time of Stephen Hales, but at the present moment there is no theory of its cause that can command general acceptance. Experiments that we have performed over several years (1925–32) have given results that were entirely unexpected at first, and that now seem to us to suggest a reason for the ill success of the existing hypotheses, and to point to a more plausible alternative.

It would probably be accepted by the great majority of plant physiologists that root pressures require living cells for their generation, and that they are propagated in the vessels of the root xylem (e.g. Pfeffer, 1900, **1**, 252; Priestley, 1920, etc.; Büsgen-Münch, 1929, p. 287; Münch, 1930, p. 177; Heyl, 1933). The first of these assumptions depends on our knowledge of the very marked influences of temperature, wound, and other stimuli on such processes, and seems established beyond all doubt. The second belief was never so well founded, however (see p. 331), and as a result of our own observations we have come to believe that root pressures may not be transmitted in vessels at all. All the more important attempts to elucidate these pressures have centred upon the problem of explaining the passage of water from the living parenchyma into the vessel cavities, and a proof that no such passage need occur would necessarily be of great importance to the theory of the subject. We have, therefore, investigated the point as thoroughly as we were able. The evidence we can bring

forward is derived in the first place from our own experiments, but to this may be added the obvious inadequacy of the evidence for the alternative assumption: each of these is treated separately in the following pages.

<center>EXPERIMENTAL</center>

Our experiments and observations were of seven kinds:

(1) Application of dye solutions to cut surfaces and bores.

(2) Introduction of solid dyes in bores.

(3) Stabbing an exposed wood surface under a solution of dye.

(4) Microscopical examination of the path of the dye.

(5) Observation of exudations at cut surfaces.

(6) Anatomical examination of the cambial zone and adjacent tissues.

(7) Measurement of exudations from cut pieces of root.

<center>(1) *and* (2) *Application of dyes*</center>

These methods have already been described in a preceding paper (Baker and James, 1933), and only the modifications necessary in applying them to roots are mentioned here. Roots about 1 cm. in diameter could easily be exposed in the neighbourhood of the stools in the sycamore plantation already described. Their course frequently lay at a few inches below the surface for considerable distances, so any required length of root could be excavated and examined. On December 23rd, 1930, such a root was exposed and severed at some distance from its aerial shoots, and the end leading away from the stool dipped immediately into a pot of 1 per cent. methylene blue. The root when cut was "bleeding" rapidly; the aerial branches had been leafless for about two months, and there was, of course, little or no transpiration stream to take up the dye. After eight days the root was excavated and cut into sections, when it was found that the dye had passed into the root for a distance of 6 ft. towards the tip. This movement, it is important to remember, was against the direction of the exudation going on simultaneously.

Another root of about the same size was treated on December 14th, 1932, in the following manner. After exposure a plasticine cup was built round it and filled with the methylene-blue solution. A sharp-pointed scalpel was then used to cut a hole passing more than half way through the root where it was immersed in the dye. The dye was removed after one hour, the root excavated and examined shortly after. The dye could be traced in the wood 13 cm. towards the root

tip and 5 cm. towards the shoots. There was, in accordance with our general experience, no dye in the bark. This root, like several others examined on the same day, was actively bleeding.

In a previous experiment on February 26th, 1925, a piece of root was cut away and fitted with a glass tube at either end, the whole being kept in a horizontal position. The tubes were supplied with a dilute solution of methylene blue, and the position of the meniscus noted in each tube. Absorption occurred at the distal (morphologically lower) end of the root and exudation at the upper in the usual manner (see p. 328), but in spite of this fact the methylene blue entered the vessels at both ends, for about 12 cm. at the lower and about 6 cm. at the upper.

We were convinced by these experiments that dye solutions may very readily enter the root xylem even when there are exudation pressures forcing solutions out of the root simultaneously. The following experiments extended the same observations to the lower regions of our stems, which also show exudation pressures in winter and spring. On January 29th, 1931, two shoots on the same stool were bored about a foot or 18 in. above the ground. One bore was rapidly filled with solid acid fuchsin and one with solid methylene blue: both were then bound up with insulating tape and left for four days. The branches were then sawn off at the base and examined the same day. In spite of a very rapid exudation and the absence of transpiration acid fuchsin had passed down the branch and so into the stump. Dye was present in all the annual rings, but apparently not in the medullary rays or bark. The dye could also be traced upwards for 50 cm. at which point the branch was still actively bleeding: the methylene blue had behaved in its branch in a similar manner. The upward movements thus demonstrated suffice to show that the entry is not caused directly by gravity as might be supposed for the more or less horizontal roots. It was also found by experiment that a head of water considerably greater than any possible slope on the roots did not measurably increase the rate of entry.

In another January experiment solid dyes were introduced into a shoot 12 ft. high at three different levels near the top, middle, and bottom of the stem. The lowest bore rapidly became filled with sap, exudation being very rapid. The dye put into this bore did not penetrate the wood to any extent, the only instance of this kind we have met with. After four days acid fuchsin from the top bore had penetrated 70 cm. up the stem and almost to the base, passing both the middle and lower bores in doing so. Methylene blue from the middle

bore similarly moved down to the base of the stem past the lowest bore, and it is to be noted that in these movements the two dyes travelled from regions where there was no appreciable exudation of sap into those where exudation was uncommonly rapid.

On February 26th six independent branches were manipulated simultaneously. Two bores were made in each, one at 120 cm. up the stem and one at 40 cm. There was no exudation at the higher level, but all the branches bled at the lower level. In three branches solid methylene blue was put into the upper bores and acid fuchsin into the lower; in the other three the dyes were reversed. The branches were cut and examined after 44 hours, by which time there was considerable penetration in every case.

TABLE I

Penetration of dye in centimetres

| Stem | Lower bore | | Upper bore | |
	Upward travel	Downward travel	Upward travel	Downward travel
1	22	21	8	18
2	14	20	15	22
3	16	18	14	20
4	10	27	16	16
5	12	16	21	22
6	14	36	31	20
Mean	14·7	23·0	17·5	19·7

The entry of the dye seems, therefore, to be quite as extensive in the presence of the active exudation as in its absence.

(3) *Puncturing the wood surface under dyes*

Mere scratching or puncturing of the wood surface under dye solution led in the summer to an instantaneous jump of the dye into the surface vessels (Baker and James, 1933), the length of the jump usually being about 7 cm. both up and down. These sudden entries are ascribable to the tensions that are released by opening the vessels, and should not be observed if the vessels contain water not under tension or under positive pressure. Twigs were, therefore, collected on November 22nd, some weeks after leaf fall, and treated in this manner with acid fuchsin. The averages of twelve punctures on three different twigs showed an instantaneous upward penetration of 1·7 cm. and a downward penetration of 1·8 cm. It seems a reasonable conclusion, that the water contained in the vessels was still under a reduced tension, though the leaves had been off and transpiration virtually

at a standstill for several weeks. These twigs were growing 3 or 4 ft. above the ground and showed no bleeding, but on December 14th a shoot was found, that showed active bleeding when sawn off 6 in. from the base. The bark was stripped away, and a number of incisions made in the usual manner under acid fuchsin. Entry of the dye occurred in all cases to a distance of 1–2 cm.; the entry was rather slower than in the previous experiments.

These experiments agree, therefore, with those of the previous paragraph in suggesting that, *when bleeding is going on, the water in the vessels, so far from being forced out under pressure, is still under tension, and sucks in additional water when the vessels are opened.*

(4) *Microscopical examination of the path of the dye*

The path of the entering dye was traced by the method already employed with the transpiration stream. Blocks of the stained wood were cut under liquid paraffin, and rather thick longitudinal sections made, leaving all the elements except the large vessels unopened. These were mounted in the same medium and examined at once, but it can be added that the distribution of the dye noted at the first examination remained unchanged after ten months. The principal localisation of the dye was found, as in the summer experiments, to be in the cavities of the bordered pits, which are restricted to the walls separating vessel from vessel. The walls themselves and the surrounding parenchymatous cells (substitute fibres, ray cells, etc.) were uncoloured, or coloured to a much lesser degree, and the conclusion again seems inevitable that the principal passage had been through the cavities of the vessels. Fig. 1 was drawn from a preparation derived from the experiment of February 26th already described. Other preparations were made from the experiments carried out on December 23rd–31st, 1930, and January 22nd–26th, 1931. They also showed the dyes, both acid fuchsin and methylene blue, retained in the pit cavities, when moving both upwards and downwards from the bore. Fig. 2 shows one of the relatively rare instances in which the dye remained visible in the actual cavities of the vessels.

In these winter experiments the dyes were applied to the wood for much longer periods than in the summer experiments. It is not surprising to find, therefore, that the walls of the vessels showed a perceptible staining that was not discernable in the summer. A further difference of greater significance was that dyes were also found in the cavities of the true fibres, i.e. those without protoplasmic or other
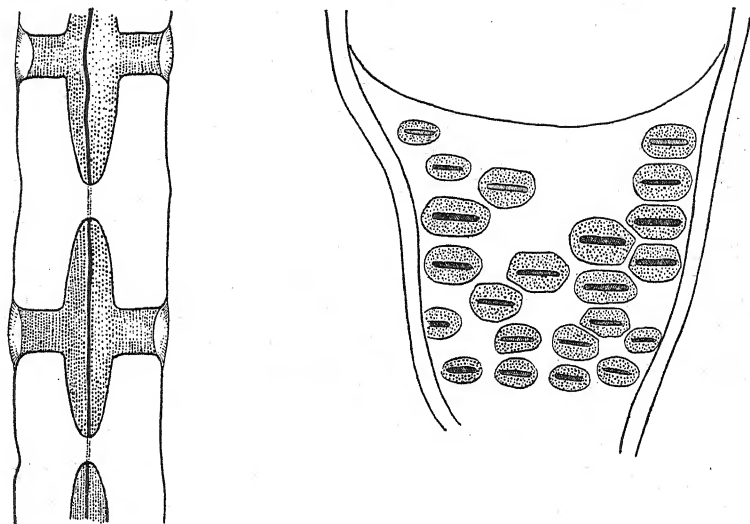
Fig. 1. Bordered pits in the walls of vessels injected with acid fuchsin (shaded); sectional view on left, surface view on right. The preparation of the section is described in the text. Drawn freehand with $\frac{1}{12}$ immersion objective and compensating ocular.
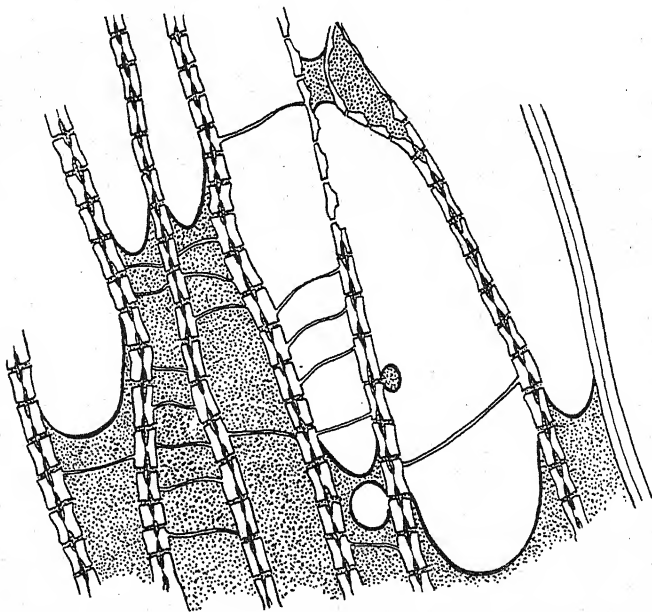
Fig. 2. Series of vessels in a radial section cut under liquid paraffin with entrapped acid fuchsin solution (shown by shading). At the lower end the vessels passed under a medullary ray. Drawn with camera lucida with $\frac{1}{12}$ immersion objective and compensating ocular. × 700

visible contents. The elements with starch grains, substitution fibres, and the ray cells remained colourless. It could be stated with confidence that many of the fibres containing dye had not been opened by the cutting, and their characteristic appearance may be represented diagrammatically as shown in Fig. 3.

We feel satisfied that the air was not introduced into these unopened fibres by the cutting, and that in the natural condition they contain water and gas under less than atmospheric pressure in midwinter. The slow entry of the dye solutions into the fibres is, therefore, not surprising once the system had been opened by boring, and brought into direct connection with the atmospheric pressure. The material of the fibre walls was not heavily stained, so it is possible that the longitudinal movement from one cell to another was by way of the fairly numerous simple pits (see Fig. 7).
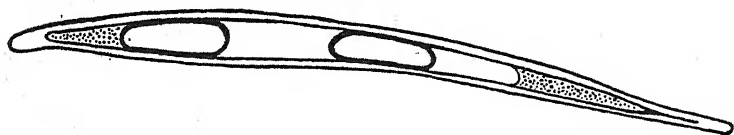
Fig. 3.

We interpret the results of the foregoing methods (1, 2, 3, and 4) as meaning that, *when exudation is going on, the water in the vessels is under reduced but still perceptible tension, and that water and gas under reduced pressure fill the fibres. The positive hydrostatic pressures associated with the exudation must, therefore, be limited to the cells with protoplasmic contents either in the wood or elsewhere.*

(5) *Observation of exudations at cut surfaces*

Stems. When an exuding stem is cut across and a clean surface trimmed with a razor, the whole surface of the wood and bark appears moist. The most abundant liquid issues, however, from the periphery of the wood, and as far as can be judged comes from the cambial zone, i.e. the undifferentiated cells and the differentiating wood and phloem cells adjoining them. The water so excreted wells over, and eventually moistens the whole face of the cut. It was not found possible to distinguish between different types of cells in these observations.

Roots. More satisfactory observations can be obtained with roots than with stems, because of the greater cross-section of their vessels, the open cavities of which can easily be seen with a hand lens at a cut surface. As in stems, the most abundant escape of sap occurs from the

tissues of the cambial zone, but here the further observation may be made that the large vessels are visibly without fluid. The great majority of them can easily be seen to have no water at the actual cut face, precisely as would be expected if their contents had been under tension when the cut was made. These observations were carried out on numerous roots and stems, and we are much indebted to Dr A. R. Clapham for confirming them. The observations of Kraus referred to more fully on p. 334, lend additional support to ours.

Further testimony is supplied by the following experiment performed with pieces of rapidly bleeding roots collected in March. The root sections were fixed vertically in a moist mixture of sand and sawdust, and a horizontal tube of narrow bore fitted to their upper (morphologically and physically) ends projecting from the sand. Roots that were not further manipulated exuded sap into the attached tubes, an advance of 22 cm., representing about 0·2 c.c., being observed in six hours. Another root was ringed, and the phloem and cortex cut away far enough to allow a rubber tube to be fitted over the cut end, and bound tightly to the exposed sides of the wood. All communication between the bark and the glass tube was thus prevented, but there was free access from the wood. In this experiment there was no exudation whatever, and similar experiments with two more roots on the following week gave similar results, one showing a slight absorption of water.

The evidence of this section suggests, therefore, that *exudation occurs from living cells, particularly those of the cambial zone.*

In an experiment rather similar to the last, but using sugar maple branches, Adams (1923) obtained a flow of sap from the wood, but did not attempt to find whether it was coming from the living or non-living elements. Experiments in which we separated the bark from the wood by pulling back the former in strips for about 2 cm. and interposing a ring of tin foil gave a slow exudation from the bark, the wood remaining moist but not actively exuding. The inevitable destruction of much of the cambial zone necessarily robs such an experiment as this of much of its value.

(6) *The anatomy of the cambial zone and adjacent tissues*

There appears to be no detailed description of these tissues, especially in roots, at the time of bleeding. As an understanding of their structure was necessary for our purposes we examined freshly collected material sectioned in alcohol and treated with iodine. The cambial zone is in most respects similar in the woody roots and lower

parts of stems, the regions concerned in our investigation. The time sequence is somewhat different, however, cambial activity being apparent in the roots for a much more extended period than in the bases of stems, in fact it seems probable from our observations that slow cambial activity in the roots continues throughout the winter (see also Priestley, 1930). The general characters of the zone in transverse section are shown in Fig. 4, which was drawn from a five-year-old stem in mid-September, when activity was drawing to a close.
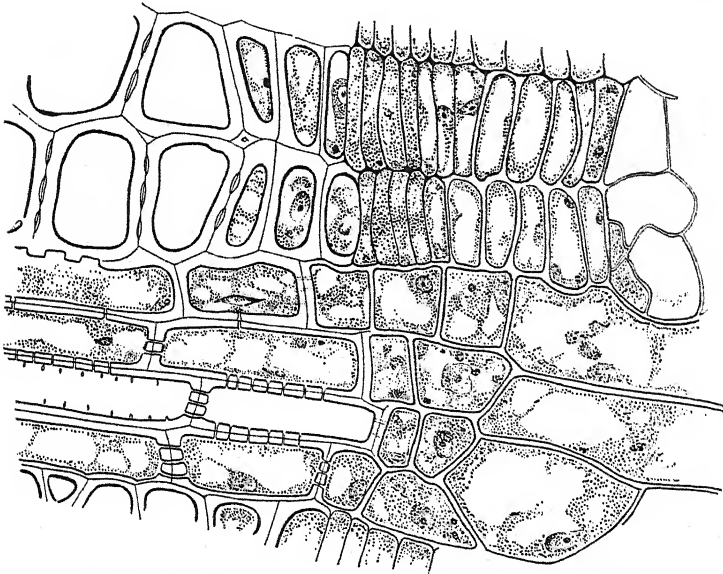
Fig. 4. Transverse section of the cambial zone near the base of a five-year-old stem. Material collected September 15th, 1930. Section treated with iodine and mounted in glycerine. Drawn with camera lucida with $\frac{1}{12}$ immersion objective and compensating ocular. × 550.

There are about five rows of undifferentiated elements in the normal cambium, but fewer at the medullary rays. Living cells, some of them forerunners of vessels, are present in the young wood, and the differentiation of sieve tubes and companion cells is evident in the young phloem. A tangential section of the youngest living wood is given in Fig. 5, and shows the differentiation of vessels with bordered pits and of true and substitution fibres with simple pits. In spite of the shrinkage of the protoplasm, connecting fibrils are visible in many places between all these kinds of elements. This very young wood is, therefore, in protoplasmic connection with the other living cells, and

until it loses its contents is an integral part of the symplast[1]. This condition does not seem, however, to persist through the winter, and at the time when bleeding is rapid the wood is fully differentiated right up to the cambial zone, though activity on the phloem side lasts much longer (see also Priestley, 1930). A small portion of the cambium in tangential section is shown in Fig. 6a. The radial walls have the usual beading except at the medullary rays, where the whole formation is different. The ray initials, as is usual, are not elongated as the other cambial cells, but are rounded and partly separated by intercellular spaces. The protoplasm of the cells approaches these spaces by simple pits (Fig. 6b) (cf. Strasburger, 1891) from which it is separated only by the middle lamella. The rays and their intercellular spaces pass both inwards and outwards from the cambial zone, and afford continuous living connection with the substitute fibres surrounding the vessels and may abut directly on the vessels themselves. Medullary ray cells and substitute fibres are interconnected by numerous large simple pits, and where they come into contact with vessels form pits of the "half-bordered" type. These are shown in surface view in Fig. 7.

The functional phloem is imprisoned between the cambium and a ring of thick-walled fibres interrupted above the principal medullary rays. The older phloem becomes much distorted and compressed, especially in the root. The young phloem contains numerous sieve tubes with narrow companion cells. There are frequent sieve plates
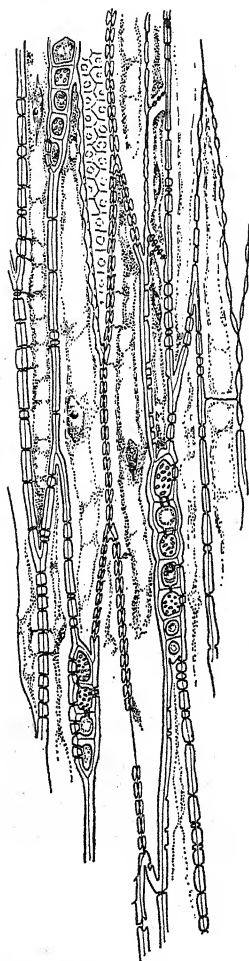
Fig. 5. Tangential longitudinal section of young living wood of stem just inside the cambium. Material collected in September. Treated with iodine and mounted in glycerine. Drawn with camera lucida, $\frac{1}{6}$ objective. × 250.

[1] Symplast has been introduced by Münch (1930) as a collective term for the living cells of a plant body all of which are regarded as linked together by protoplasmic fibrils of high permeability. Those cells excluded from this organisation are distinguished as apoplast.

usually oblique, as shown in Fig. 8, and generally radially orientated; there are also occasional lateral sieve plates where sieve tubes run side by side. Particular attention was paid to the condition of the young phloem in winter. It is commonly believed that in certain plants, especially *Vitis*, the sieve plates become plated and their pores blocked with deposits of callus in the winter months. It is not clear (cf. Hill, 1908) that this applies to all the sieve plates even in those species in which it occurs, and such species are probably not numerous (Haberlandt, 1914, p. 330). Priestley (1930) thinks it very
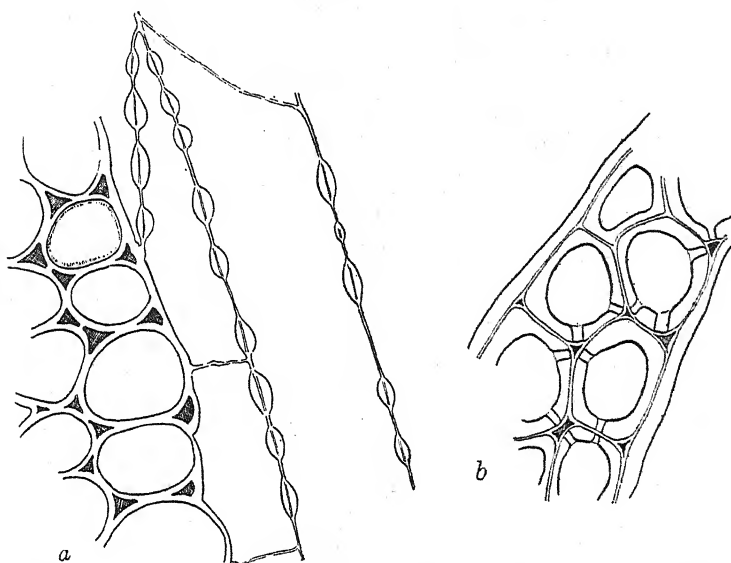
Fig. 6*a* and *b*. Tangential sections showing medullary rays in the cambium. The intercellular spaces are shaded. Drawing, etc., as Fig. 4.

doubtful whether the phloem of woody organs in general shows any marked cessation of activity in winter, and our own observations on sycamore roots support this view. We examined the condition of the phloem in December in a series of preparations from roots that had begun to bleed. Cambial activity was well marked, the undifferentiated zone being seven to nine cells wide, and the bark stripped only too readily from the wood when sections were attempted. There was a fairly wide zone of young phloem in which the sieve tubes were clearly functional, i.e. had thin protoplasmic linings, and open pores to the sieve plates. Small starch grains were visible in the protoplasm of the sieve tubes, but in the phloem starch is mainly confined to the

rays. Large and numerous grains are found in the medullary rays, substitute fibres and parenchyma of the young wood, as well as in the cortex, a condition similar to that observed by Swarbrick in apple branches (see Priestley, 1930).
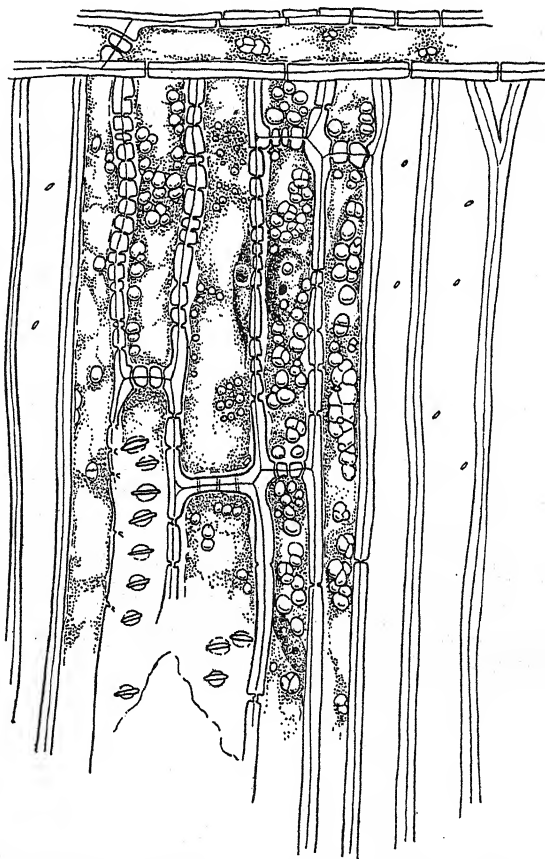
Fig. 7. Radial longitudinal section of stem showing sheath of living cells round a vessel. Preparation and drawing as Fig. 4.

(7) *Measurement of exudation from cut pieces of root*

A number of experiments were performed on pieces of root about 1 cm. in diameter, that were excavated, wrapped in wet cloths, and immediately brought to the laboratory. They were set up in various ways that allowed the exudation to be closely observed, and the following selection indicates the results obtained.

On February 19th, 1925, a straight piece of root 37 cm. long and 0·8 cm. diameter was attached at each end to a piece of narrow-bore tubing and buried in a mixture of sand and sawdust. The tubing rose at each end 25 cm. above the root and was then continued in a long
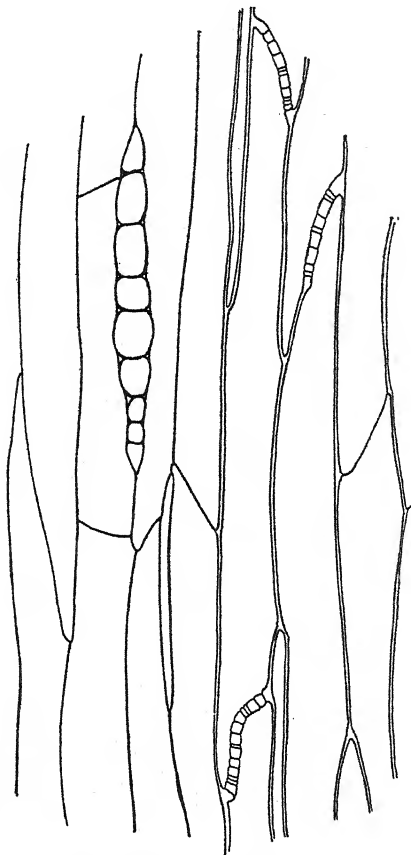
Fig. 8. Tangential section of root in the young phloem. Material collected January 21st, when the root was bleeding. Section treated with iodine and mounted in glycerine. Drawn with Watson projection apparatus. ⅛ objective. × about 300.

horizontal limb. The sand mixture was kept moist and its temperature maintained between 16 and 18° C. Exudation from the morphologically upper end began almost at once and absorption at the lower end simultaneously. The movements of the menisci in the horizontal tubes are recorded in Fig. 9. The bore of the glass tubing was 5 mm.,
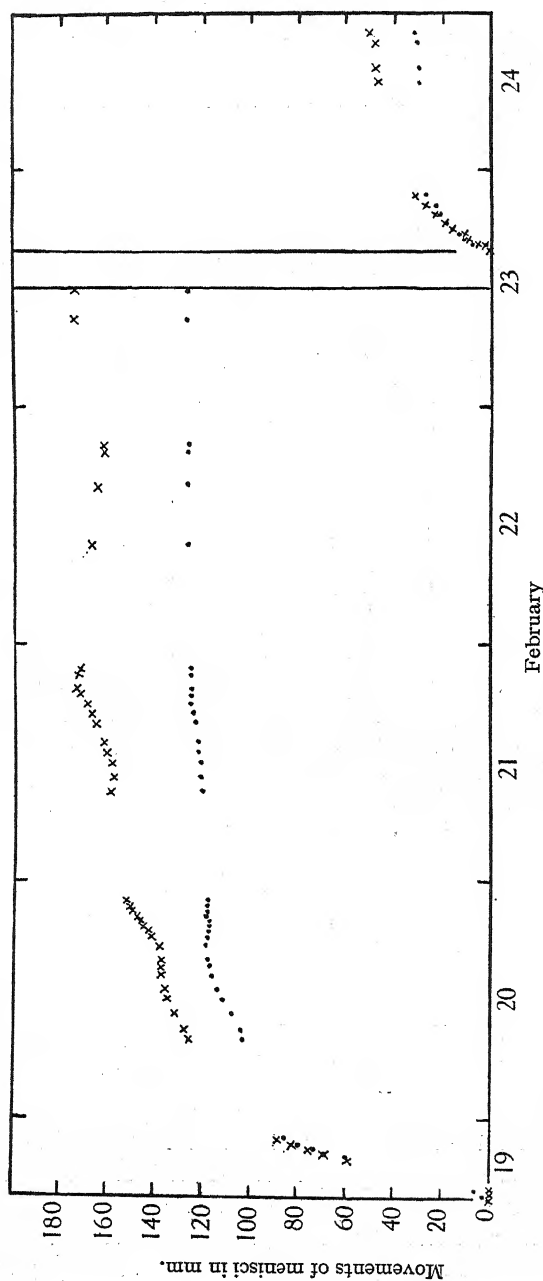
Fig. 9. Exudation (shown by dots) and absorption (shown by crosses) of a piece of sycamore root. At the break fresh surfaces were cut and the experiment set up again. Details of the experiment in the text.

hence at the end of the period 34·2 c.c. ($\pi \times 0.25^2 \times 17.4$) of water had been absorbed, and 24·9 c.c. ($\pi \times 0.25^2 \times 12.7$) exuded: 9·3 c.c. had been retained by the piece of root whose total volume was 29·6 c.c. We may safely assume that the bulk of the retained water had passed into the wood fibres as in the normal filling up of the tissues during the winter months. The most important point illustrated by this experiment is, however, the polarity of the root's action; water being taken up by the morphologically lower end, and excreted at the upper end. This result was often repeated with other roots and only once failed.

The important point emerges from the experiments of this section that *the exudation is always in the morphologically upward direction, even when the vessels have been opened at both ends, and will allow water to pass freely in the longitudinal direction.* A drop of water placed at the physically upper end of the piece of root immediately causes a drop to appear at the lower end. If the position of the root is reversed the drop at once appears at the end that has now become the lower. That root pressures should appear in a system such as this is, of course, incompatible with some commonly accepted theories concerning the mechanism of the process.

EXAMINATION OF THE BELIEF THAT EXUDATION IS FROM THE VESSELS

At the present time it is a tacit assumption that root and other exudation pressures, though they may depend on living tissues for their generation, are transmitted in the vessels of the xylem. So general is this assumption that a theory such as ours cannot afford to pass over it in silence, and it is necessary to discover the evidence on which it was originally founded. The publication which has probably had the most influence on our ideas about the plant's water economy in recent years is Maximov's *Plant in relation to water* (1929). It will be interesting, therefore, to use this as a starting-point for our search. Maximov offers no personal experience on the point, but in speaking of root pressure refers to the well-known papers of Priestley in this journal (1920, 1922; Priestley and Armstead, 1922), to Atkins (1916), and to Pfeffer (1900). Priestley in his turn starts by saying "Most of the data presented below are drawn from the work of other investigators. In these papers, therefore, he is more concerned to marshal experimental facts already known than to discover new ones, and speaks on his first page of the "process by which sap is

driven up the xylem from the roots," and later of "a hypothesis to overcome the difficulty of the passage of water from the cells bordering on the xylem vessels, into the xylem vessels." Nowhere in the papers quoted have we been able to find any experiment designed to show that the difficulty arises, i.e. that water is in fact driven into the vessels. Atkins (1916, p. 201) also takes the matter for granted: in a paragraph beginning "We may imagine…" he goes on to explain that given a relatively high osmotic pressure in the tracheae, and a continuous semipermeable membrane round them contributed by the circumvasal parenchyma, a flow of water from soil to vessel would result. There is no attempt or intention to demonstrate that such a flow exists.

We thus come to Pfeffer to whom, indeed, all roads lead in this territory, his consideration of exudation pressures having been the basis of all subsequent work. Even here, however, we are without first-hand evidence about the path of the sap. Pfeffer (1900, p. 258) merely makes the statement "The sap exudes from the xylem, and especially from the tracheae and tracheides," and for its substantiation refers us to Schwendener (1886 and 1892), Strasburger (1891), and Brücke (1844). It is a little surprising to find, therefore, that in the place cited (1886, p. 222 of collected edition) Schwendener merely says that it is well known that an upthrust worth mentioning is present in the vessels only during the bleeding period ("Andererseits ist bekannt, dass ein nennenswerther Auftrieb von unten d. h. von der Wurzel her, in der Gefässen nur während der Zeit des Blutens vorhanden ist"). He offers no experimental evidence, and refers to no previous authorities. Speaking of the period immediately after bud break, he adds that the bleeding takes place not from the vessels but from the tracheids and libriform cells ("Ueberdies bluten auch die tiefer gelegenen Schnittflächen oder Bohrlöcher eines Stumpfes offenbar nicht aus den Gefässen, sondern aus den saftreichen Tracheiden und Libriformzellen"). The evidence for the latter statement was, however, merely that water free from air bubbles could be collected from woody stumps in spring by introducing tubular bores. His distinction rested, that is to say, on the now discarded belief that the vessels contained a good deal of air at such periods.

Strasburger does not pretend to offer experimental evidence on the page cited by Pfeffer (Strasburger, 1891, p. 859), nor, as far as we have been able to discover, on any other. He merely remarks that some of the living cells adjoining vessels are pitted towards the tracheae and appear to lose their reserve materials more readily than those that

are unpitted. He adds: "Nur die mit den trachealen Bahnen so (i.e. by pits) verbundenen Elemente durften an dem Zustandekommen des Blutungsdruckes betheiligt sein, wie auch anzunehmen ist, dass sie allein darauf eingerichtet sind, den trachealen Bahnen Wasser zu *entziehen*" (our italics). He makes it clear on p. 835 that he believed water to be taken up by the osmotically active parenchymatous cells from any or all of the surrounding elements, vessels included, and pressed out along the line of least resistance. Purely from anatomical considerations he supposed this path to lie through the membranes separating the circumvasal parenchyma from the vessels, and so along the vessels themselves. There is no question of direct observation or experiment.

Brücke's paper (1844) is on a different footing. He says that when vine (*Vitis vinifera*) branches are cut across during the bleeding period sap can be seen to well out of the cut ends of the large vessels quite clearly with a lens and sometimes even with the naked eye. Cuts made immediately before bleeding began showed the vessels to contain "nothing but air," i.e. according to modern notions the water was under tension and had retreated on cutting. All the other elements were saturated with water, and this Brücke took to mean that the primary rise of sap was not by way of the vessels, a very interesting conclusion when compared with the arguments of our last section (pp. 318 *et seq.*). For confirmation of his first observation Brücke refers to Dutrochet and Rominger. Dutrochet (1837, p. 369) was rather guarded in his statements, which again applied only to vine: "...c'est exclusivement de ces gros tubes que la sève paraît sortir." He adds that in detached pieces of twig water could be seen rising and falling in the large vessels when the twig was bent, but that it was impossible to make corresponding observations on other elements merely on account of their smallness. He did not believe the sap to rise through the vessels, but through the fibres. Rominger (1843) also made his observations on vine branches, and considered that the sap was exuded from the vessels. He rather strains the reader's credulity by stating that he could see "two lines" ($\frac{1}{6}$ inch?) into the cut vessels, and measured this distance by inserting a needle until he could no longer perceive the point. We were quite unable to repeat this on vines growing in Oxford. Rominger was somewhat disturbed by the fact that occasional air bubbles were also produced from the vessels, realising that this was rather surprising if the vessels had contained water under pressure from the start. Kraus (1882) watched numerous detached pieces of vine branches, stocks, and

roots, embedded in wet sand. He obtained plentiful exudations in all cases, but found that it never came from the vessels. In his experiments the pieces of material were usually about 6 cm. long, and the vessels were opened at both ends, so it might be supposed that any extrusion into them found its way out in the downward direction. In our own experiments with sycamore, where both ends of detached pieces of root were kept under observation, no such backward exudation took place, however.

Our backward trail from Maximov's monograph ends abruptly at this point. As far as we can discover, the only other direct observer, who managed to satisfy himself that exudation occurs from vessels, was Sachs (1873, p. 594, 3rd ed.). He did not claim that exudation was invariably from the vessels, but says that the sap "bei Monocotylen aus den Xylembündeln der einzelnen Stränge hervorquillt, und dass es vorwiegend aus den Oeffnungen der grösseren Gefässe kommt." There is no corresponding statement for the dicotyledons, and the only monocotyledon mentioned in his list is *Zea mais*. It is, moreover, only by implication, not from direct statement, that one assumes that Sachs personally conducted the experiments he describes. Kraus (1883) endeavoured to confirm the statement with several genera of grasses, *Avena*, *Panicum*, *Zea* itself, and *Sorghum*. He says that owing to their structure it is difficult to locate the exact position of exudation in these stems. The principal localities appeared to him, however, to be the phloem and to a lesser extent the fibrous layer encircling the whole bundle. We have ourselves made almost identical observations with the dicotyledon *Impatiens* (garden hybrid). When the stems were severed near the base by a razor, flooded with methylene blue the xylem was immediately picked out in bright blue, while a drop of sap collected rapidly over each bundle, appearing to come particularly from the phloem and peripheral tissues.

Kraus (1881, 1882, 1883), it seems safe to say, is the only person who has ever carried out any extensive series of observations to determine the tissues from which exudation occurs, and his results have been ignored by two generations of botanists, probably, and not altogether undeservedly, on account of his extreme prolixity. He examined more than sixty different woody and herbaceous species, using both detached pieces and complete root systems, appears to have been thoroughly alive to the difficulties of the undertaking, and states his eventual conclusions with caution. He found a great deal of variation between species, different parts of the plant and so forth. In some plants he found any fine discrimination impossible, and says

so; but about others he is more certain. There was only one type of element, which he never definitely found to be exuding sap, and that was the vessels. All others, including even collenchyma, xylem parenchyma, and fibres, he thought he could see exuding at one time or another.

It is thus clear that, although a possible exception may have to be made for vine branches, see p. 333, there is no sufficient evidence to justify the assumption that the exudations usually well out of the vessels.

Earlier workers have also recorded isolated observations hinting at the existence of tensions in vessels at the time of exudation, but have never placed this interpretation upon them or troubled to follow them up. Schwendener (1886), for example, says (p. 223, footnote) that while some bores that he made in the lower regions of beech trunks showed exudation pressures, others near them at the same level showed suctions, and we may suppose that pressure or suction resulted according to the chances attending the penetration of his pointed metal tubes. The similar observations of MacDougal (1928) on coniferous trees are vitiated by the fact that his so-called exudation pressures were probably due to the extrusion of resins (see Münch, 1930, p. 174). Hartig (see Büsgen-Münch, 1929, p. 289) has reported that measurable root pressures are not developed by conifers. Jones, Edson and Morse (1903; quoted from Miller, 1931, p. 677), investigating the sap flow of sugar maples, instead of recording the steady positive pressure they appear to have expected at times measured suctions as high as six atmospheres during the period of flow. The common observation of many workers that a "latent" period precedes the extrusion of sap is likely to be due to a soaking up of the first extrusion at the cut surface of the wood. We have ourselves observed this to happen in sycamore roots.

DISCUSSION

The path of the sap and the mechanism of the pressure

The preceding pages point clearly to the fact that positive hydrostatic pressures do not exist unless rarely in vessels at the time when active exudation is going on, and further that there is no adequate evidence for supposing that the liquid escaping from cut surfaces normally issues from the vessels. Experiments have been described in which dye solutions have passed steadily into the vessels, and

slowly into the xylem fibres while exudation has been going on, and we are forced to the conclusion that any push on the sap up to the cut surfaces must be transmitted via the living cells. We were further able to satisfy ourselves that the plates of young sieve tubes had open pores when bleeding could be caused, and that the development of young living wood cells probably takes place only after bleeding has begun.

In view of these facts it seems preferable to suppose that root and stem exudation pressures are transmitted, not through the vessels as usually assumed, but through the living symplast, and that any movements of liquid due to them take place principally through the sieve tubes. The underlying principles, the requirements, and the application of such a system to the problems of translocation, have recently been given by Münch (1930) in a very thorough fashion. The basis of the mechanism suggested by Münch lies in the fact that when two osmotic pressures are caused to operate against one another by way of a liquid connection, movement of solution occurs from the direction of the greater pressure towards that of the lesser pressure until the movements of solution have equalised the osmotic activities at the two ends of the path. Simultaneously water will be taken in under the influence of the greater pressure and thrust out against that of the lesser.

In applying this principle, instead of regarding each living cell as an osmotic unit, the whole aggregation of living cells, the symplast, is considered as being linked together by plasmatic fibrils into a single osmotic system. The point in this system at which the concentration of osmotically active substances is temporarily highest will develop relatively high osmotic and hydrostatic pressures, and if external water is available liquid movements will be set up from this position towards any other where pressure is lower. There will necessarily be an excretion of water, or, if the membrane is not completely semipermeable, of water containing some solute, at the position towards which movement of solution occurs. Supposing the osmotic pressure of the symplast to be highest at the lower end of the piece of root, the tendency will be for water to be taken in at this end, and for solution to travel upwards through its cells. Simultaneously water or solution must be given out or stored somewhere to correspond with the water taken up. If a cut has been made this fluid naturally escapes from the exposed surfaces of the symplastic cells until they callus over or become blocked in other ways. If the root is intact there is no such easy escape; consequently the back pressure prevents such extensive

movements, and the only movement that takes place is associated with the filling up of the higher regions that has so frequently been shown to occur at these periods (Hartig, 1882, 1883; Craib, 1918, 1920, 1923). This filling up seems to consist mainly of passing fluid into the partially empty fibres, but distensions of the living cells, and to a limited extent reduction of the tension in the vessels, may also occur.

It is an obvious necessity of this scheme that the path of least resistance to the fluid movements should be within the symplast. Living cells come into contact with the vessels over their entire surface, and it has always been assumed that passage into the vessels will be easy in consequence. A little reflection will show, however, that this is not necessarily the case. When transpiration is rapid very considerable hydrostatic tensions, up to 30 atmospheres (Renner, 1911, 1912; Dixon, 1914, 1924), are developed in the tracheae, but at the same time positive hydrostatic pressures exist in the living cells surrounding them. A great part of this pressure difference must be maintained across the single membrane layer separating the living cells from the tracheae, since frictional resistance in the wood is not very great (cf. the easy passage of water through vessels, p. 331), and it is only the very great forces developed during transpiration that suffice to drag water through it. Were there not a considerable resistance at this point, either the tensions would be released, or the cells surrounding the vessels would become flaccid instead of containing water under pressure. The resistance does not, of course, depend solely on the friction encountered by the water molecules in threading their way through the pores of the membrane, but also on the whole balance of osmotic pressures acting at the time. In assessing the net pressure directed against the pull into the vessels the pressures acting simultaneously throughout the symplast must also be taken into account.

Atkins (1916) has shown that the osmotic pressure of the living cell sap is always higher than that of the tracheal sap, hence there will be a tendency for water to be withdrawn from the vessels across the semi-permeable membranes of the surrounding cells and an easier line of escape for the moving fluid will be through the sieve tubes, and perhaps through the protoplasmic connections of other living cells, since such movements do not involve the penetration of surface membranes. The amount of water actually withdrawn from the vessels is likely to be small, since there is an easier uptake from outside the root. Cut pieces are capable of taking this water at the exposed surfaces, but in an intact root water will be acquired by way of the root hairs.

A second requirement of the theory is that sap movement should take place from a region of high osmotic pressure to one of low pressure, i.e. we should expect to find the osmotic pressure of the cell sap decreasing from the root into the base of the stem when this is being filled up. The converse proposition is known to be true of translocation, i.e. movement of sap occurs from leaves having high osmotic pressures into cambia, growing points, etc., with relatively low ones (Münch, 1930). A few data are also available bearing on our own problem. The results of Atkins (1916) relating to *Acer* spp. show that the living cells of the wood always contain a much more concentrated sap than the tracheae, but that the two concentrations appear to run parallel. The tracheal saps of *Acer macrophyllum* in October show a minimum concentration at 4 m. above the ground-level, with an increase both upwards from this point and downwards from it into the roots. The results obtained from *Acer pseudoplatanus* for the total sap including the living cells are unfortunately less complete, but show that the root sap was always more concentrated than that of the stem as high as 8 m. above the ground. Seasonal variations are very considerable, particularly in the stem, and in *Acer pseudoplatanus*, *Populus*, *Fagus*, and *Salix* spp. the tracheal saps of stems showed a very great reduction of concentration in the autumn, bringing it below that of the roots which remains relatively constant. This is in sharp contrast with the summer condition when translocation is in progress. Walter (1931) collects a large number of annual records of osmotic pressures of the aerial parts of plants of widely different species growing in widely different situations, and calls attention to the minimum that always occurs at the time of bud break. A similar minimum was shown in February in the living cells of the bark and cortical tissues of *Populus tremuloides* trunks (Lewis and Tuttle, 1920).

From the existence of a region of low osmotic pressure towards the base of the stem it would appear that there are two principal hydrostatic pressures present in the symplast at the time of bleeding, the upward thrust from the roots and a downward thrust from above. The exact position and extent of the region of low pressure varies to some extent, no doubt, with circumstances; it may, for example, rise as bleeding proceeds. This region is of great interest, because it is here, according to our mechanism, that water will be pushed across the semi-permeable membranes against the relatively weak osmotic pressures that characterise it; in other words it is here that liquid will be pressed into the non-living vessels and fibres from the sym-

plast, and lead to the gradual release of tension in the vessels and the filling up of the fibres already mentioned.

The vine is of special interest in this connection, since it appears (see p. 333) that water penetrates into its vessels much more extensively than in other plants, and may in aerial branches at least be exuded from the vessels at cut surfaces. This, we think, is to be regarded as an unusual condition due, perhaps, to the fact that the vine is one of the few plants whose sieve plates are callused in the bleeding period. The callusing leads to an unusually high resistance to movement in the symplast, and hence the line of least resistance in this plant does lie across the semi-permeable membranes and along the vessels. This explanation has the further merit that it explains the great differences of concentration of solids in the exudates from different plants that has been observed (e.g. Pfeffer, 1900). Whereas the vine exudate has a very low concentration of total solids, 0·156 per cent., including only 0·0008 per cent. of sucrose (Wormall, 1924), the *Acers* have more than ten times this total of sugars alone, the great bulk being sucrose. The following figures serve as illustration: *Acer platanoides* 1·15–3·71 per cent. sugars (Schroeder, 1869), *A. saccharinum* 5·15 per cent., *A. rubrum* 2·81 per cent., *A. negundo* 2·40 per cent. (Wiesner, 1928, **2**, 2101). In the vine we may, therefore, assume that the bulk of the exuding sap represents water that has been pressed through the semi-permeable membranes into the vessels and in the *Acers* that we are dealing with the true symplastic sap exuding direct from the cut cells of the cambial zone (young phloem, etc.). In both cases the fundamental mechanism is the generation of unequal osmotic pressures in the symplast and the consequent movement of water in the direction of least resistance. To what extent leak into the vessels occurs in any given species can only be determined by direct experiment; in vine and sycamore we appear to have the two extremes. Other species may resemble either or show intermediate effects.

Exudation and translocation

The sap movements caused by the "bleeding" pressures developed in roots and the lower regions of stems present numerous features in common with translocation: both feed the cambium, involve positive pressures, and move a sap particularly rich in sucrose, and one of the most attractive aspects of the theory given above is that it brings these related phenomena under a single heading. The motive force in all these cases is probably osmotic, and it is trans-

mitted hydrostatically. Since movement of sap occurs from regions of high osmotic and consequently high hydrostatic pressures, the direction of flow at any moment depends on the relative positions of high and low pressures. The normal positions and the consequent directions of flow during translocation have already been mentioned, and the artificial manipulations of internal osmotic pressures carried out by Münch have led to the expected reversals of sap flow. The phenomena of root and exudation pressures in the lower regions of stems may be regarded as a natural reversal of the translocation conditions. The upward movements associated with them occur when the leaves are off, and the downward pressures consequently reduced to a minimum. At the same season there is a continuous hydrolysis of reserve materials, especially starch, in the roots, and this supplies the necessary continuous supply of osmotically active substances, mainly sugars and especially sucrose. Leclerc du Sablon (1905) showed that the carbohydrates in the roots of young pear trees fell from 30·3 per cent. of the dry weight on February 18th to 22·4 per cent. on April 13th. The results of Atkins (1916, see above) seem to imply that the reversal of movement is brought about by a reduction of the pressures at the upper end of the path and only to a much slighter extent by an increase at the lower: the fluctuations are clearly due to fluctuations of carbohydrate, not electrolyte content. The high osmotic pressures are associated with the formation of sucrose, and we found in harmony with these results that our sycamore exudates contained abundant sucrose, since they reduced Fehling's vigorously after hydrolysis though not before, and coloured Seliwanoff's solution red. They also showed well-marked diastatic activity, but no invertase or hexose[1]. Microscopic examination of our isolated pieces of root showed that starch disappeared practically completely during prolonged periods of bleeding after isolation, and that the starch of the cortex, phloem, and younger wood was the first to go.

Hydrostatic pressures are not necessarily absent from the upper stem at these periods, but merely overweighted by the upthrust from below. Given a supply of water to the upper end one might expect a shoot to excrete "backwards" from the cut stump provided that the osmotic pressures were greatest above as appears to be the case (Walter, 1931; Maximov, 1929), both during the bleeding period (Atkins, 1916) and in the summer.

This indeed only amounts to the common condition of trans-

[1] We are indebted to Dr M. Cattle for the enzyme data. The method was to estimate reducing sugars by the Hagedorn-Jensen micro-titration.

location with an artificial supply of water to take the place of the normal supply cut off by detachment of the stem, and as long ago as 1878 Pitra realised this condition experimentally. We thus see the essential unity of all sap movements in the plant other than those of the transpiration stream. Only one distinction seems necessary, that between movements under pressure and movements under tension, the former occurring in the symplast and the latter in the non-living apoplast. The pressure is, perhaps, always of the same nature, whether it gives rise to bleeding, filling up of a trunk, or translocation, that is to say, it is a hydrostatic pressure acting upon cell saps. "Root," "exudation," and "translocation" pressures are, therefore, all included, and the common name of *sap pressure* might well be used.

Summary

1. Experiments are described showing that sycamore roots and stems take up dye solutions readily even when actively bleeding.

2. Microscopical examination showed that the dye had passed along the vessels and also into the non-living fibres.

3. Direct observation of cut surfaces showed that the sap exuded did not come from the vessels, but out of living cells, particularly those of the cambial zone (cambial region including the differentiating tissue mother cells).

4. Anatomical examination of the cambial zone showed that young sieve tubes were present with open sieve plates at the bleeding period.

5. Measurement of the amount of exudation and absorption at both ends of cut pieces of root showed that although the vessels were completely opened exudation occurred only at the morphologically upper end, while absorption exceeding the amount of exudation occurred at the lower.

6. An examination of the literature is given to show that, making a possible exception for vine branches, there is no adequate evidence to demonstrate that exudation takes place from vessels. There are, on the other hand, isolated observations that may be interpreted as meaning that the water in the vessels is under slight tension even while bleeding is going on.

7. The experimental and other results of the above sections are taken to mean that such tensions do exist in vessels, and that the positive hydrostatic pressures associated with bleeding and exudation exist in the living cells (symplast), and that the sap movements due to them take place principally in the phloem.

8. Upward movement is considered to be due to the relatively high osmotic pressure in the roots which leads to high hydrostatic pressure when water is gained by way of the root hairs or a cut surface. When the system is opened by cutting, any extrusion of sap will necessarily be from the living cells. In the natural closed system water is forced across the semi-permeable membranes into the non-living fibres and vessels at the place or places where the osmotic pressures of the living cells is lowest. This usually lies a short distance up the stem, the osmotic pressure rising again above this point. If there is a suitable supply of water, downward movement towards this point will set in along the phloem as well as the upward movement from the root.

9. If the extrusion of water into the non-living cells is unusually vigorous and movement in the symplast specially restricted (as may be true of *Vitis vinifera*) exudation will also occur from vessels when cuts are made.

10. The details of this theory and its implications are discussed and shown to harmonise with the results of other workers.

11. The probable similarity between the pressures involved in exudation and translocation is pointed out, and the term sap pressure suggested as a common designation.

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THE NOEGGERATHIAE AND TINGIAE

THE EFFECTS OF THEIR RECOGNITION UPON THE CLASSIFICATION OF THE PTERIDOPHYTA: AN ESSAY AND A REVIEW

By ISABEL BROWNE

(With 2 figures in the text)

TOWARDS the beginning of the present century it was customary to divide the Pteridophyta into four phyla, Equisetales, Sphenophyllales, Lycopodiales and Filicales ((23), p. 497), or to include the first three in the main group of the Lycopsida and contrast this group with the corresponding one of the Pteropsida, represented by the Filicales ((9), (10), p. 242). Subsequently the Psilotales, hitherto usually associated with the Sphenophyllales or Lycopodiales, were separated as a distinct phylum ((26), pp. 396–8), while the emergence of the Devonian group of the Psilophytales further increased the number of main groups or phyla. In 1927, Dr Hirmer in his *Handbuch der Paläobotanik*, divided the Pteridophyta into six groups ((7), p. 146). First come the Psilophytales, regarded as relatively primitive forms from which the other groups can be at any rate morphogenetically derived ((7), p. 692); then the Lycopodiales; then the recent Psilotales; then the Articulatales, containing the types previously included under the Pseudoborniales, Sphenophyllales and Equisetales, as well as the Protoarticulatae (a small Devonian group, our knowledge of which is chiefly due to Drs Kräusel and Weyland, cf. (13) and (14)). A fifth phylum, that of the Cladoxylales, contains but a few forms, *Cladoxylon* Unger, with half a dozen species, and probably *Völkelia* Solms Laubach, while the sixth phylum is that of the Filicales.

In 1931, Dr Němejc, while retaining Dr Hirmer's six groups, suggested the formation of a seventh, co-ordinate with them, that of the Noeggerathiales, to include certain fossils, originally described as ferns or cycads, but in which, as the result of the work of the last seven or eight years, we are beginning to recognise a new group (19). In the present article it is proposed briefly to consider the effect on our conception of the interrelationships of the Pteridophyta of the recognition of this new group. The Cladoxylales and Filicales

will be left out of consideration, as the Noeggerathiales appear to be extremely remote from these two phyla.

The Noeggerathiales are known only as impressions or incrustations. They appear to have been a Permo-carboniferous and Triassic
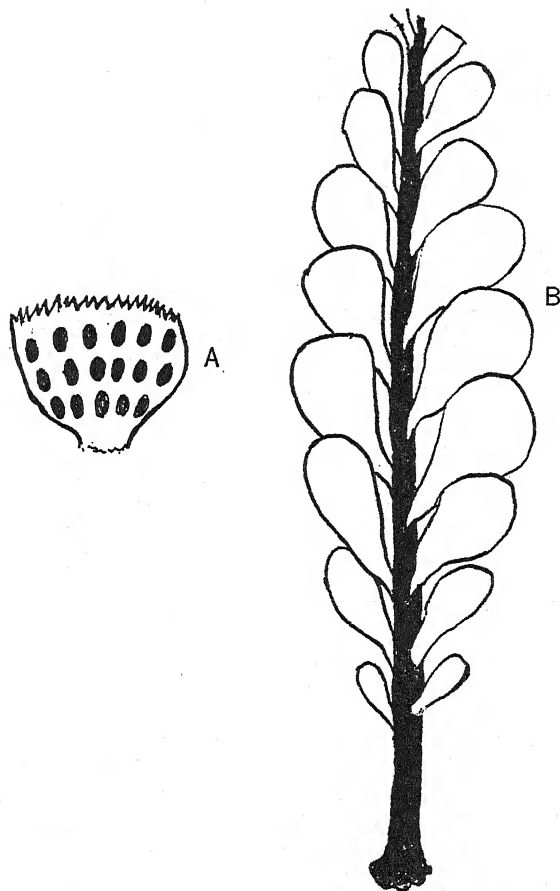
Fig. 1. *Noeggerathia.* A. Fertile leaf, seen from the upper (inner) side. Sporangia black. Highly diagrammatic and natural size. B. Shoot, the axis has been coloured black to make the insertion of the leaves clearer. Reduced. A original, B modified from Seward ((27), p. 429, Fig. 302 A), after Stur.

group characterised by the possession of flattened shoots, bearing two or four rows of simple leaves, each of which receives several veins that dichotomise once or twice, the resulting veins running unbranched to the margin of the leaf. Where, as in *Noeggerathia* and

Plagiozamites, there are two rows of leaves, these are inserted slightly obliquely on the axis and spread out in one plane (Fig. 1, B). The shoot thus simulates a simply pinnate leaf, and this resemblance is heightened by the fact that the leaves tend to increase somewhat in size and to vary a little in form as we pass upwards towards the middle of the shoot, and then to decrease again towards its apex. In *Tingia* (4, 5) the leaves are in four rows and those of two of the rows are smaller and slightly more deeply cut than those of the other two (Fig. 2, C, D). Though the difference in size between the two kinds of leaves is not great, only the larger are usually visible, because the rock always splits along their plane of expansion, which is similar to that of the two rows of leaves in *Noeggerathia*. It was Dr Halle who, byre moving parts of the matrix and exposing the smaller leaves of *Tingia* (of which a single specimen had previously been described as a fern leaf), cleared up the morphology of that genus and, in 1927, pointed out that the only forms which might be "at all closely related to it" were *Noeggerathia* and *Plagiozamites* (5). As early as 1910, however, Professor Seward had pointed out that *Noeggerathia* was not a leaf, but a flattened shoot ((27), pp. 429–31), and in 1917 he had expressed doubts as to whether *Plagiozamites* was a true representative of the Cycadophyta ((28), p. 529). It seems to me that to judge from Renault's figure and description of his *Sphenozamites Rochei*, this plant may prove to be a *Plagiozamites* (cf. (20), p. 327 and Pl. LXXXI, fig. 1).

No fructifications have as yet been recognised as belonging to *Plagiozamites*. For *Tingia* Professor Kon'no has described a single type of cone, associated with two species of the genus (12), while in the case of *Noeggerathia* three forms of fructification have been described: that of *N. foliosa* Stbg. (described as *Noeggerathiostrobus bohemicus* Weiss before it was found in connection with a vegetative shoot); that of *Noeggerathia zamitoides* Sterzel; and one which it has not been possible to assign to a definite species and which is known as *Noeggerathiostrobus vicinalis* Weiss.

We owe our knowledge of the fertile parts of *Noeggerathia* chiefly to the late Dr Sterzel (29) and to Dr Němejc (18). Dr Sterzel's paper appeared before Dr Halle's work on *Tingia*; and Dr Němejc's paper (18) was already in print when he became acquainted with Dr Halle's elucidation of the morphology of *Tingia*. Both authors described the shoots of *Noeggerathia* as pinnate leaves and the fructifications as though they were the fertile portions of the leaf of a fern or a cycad. In summarising their descriptions I shall employ a terminology in accordance with our present conception of the fructi-

fication as a cone, a conception which has been unreservedly accepted by the survivor, Dr Němejc, in a recent publication ((19), p. 113).

The best known of these cones is that of *Noeggerathia foliosa* Stbg., which was borne terminally on an ordinary vegetative shoot. The sporophylls were shorter and relatively broader than the ordinary leaves, being from 2 to 2·5 cm. broad and only 12–15 mm. long. They were much narrowed at the base and were inserted obliquely on the axis, just as were the ordinary leaves. Each sporophyll has several main veins, which fork once or twice, the resulting veins running more or less parallel to each other and each terminating in a tooth of the broad distal end of the sporophyll. On their upper surfaces these sporophylls bear sporangia—estimated at about 17—arranged rather regularly in longitudinal and transverse rows. The cone was heterosporous and the sporangia are externally indistinguishable. The megaspores were from six to nine times as large as the microspores and were present in far smaller numbers in the sporangium. Much less is known about *Noeggerathiostrobus vicinalis* Weiss. The cone and sporophylls were rather larger; the sporangia on a sporophyll seem to have been rather more numerous, but were arranged in the same longitudinal and transverse rows. According to Sterzel, the cones of *Noeggerathia zamitoides* were inserted spirally on a relatively main axis, about 10 mm. thick. The sporophylls were of much the same nature as the leaves and were similarly arranged in two rows. Their venation bears a general resemblance to that of the sporophylls of *N. foliosa*, and they, too, bear on their upper surfaces a number— probably a rather smaller number—of sporangia arranged in longitudinal and transverse rows. Possible spores, said to be about 0·5 mm. in diameter, have been found, but only outside the sporangia.

The cones of *Tingia*, known as *Tingiostachya tetralocularis* Kon'no, are borne in pairs on a bifurcating branch. Below the cone, which consists of tetramerous whorls of free sporophylls, the axis bears numerous, often deeply split leafy scales. Each sporophyll has on its upper surface a large tetralocular synangium, which is situated a little way out (Fig. 2, A, B). Beyond it the sporophyll turns upwards. Professor Kon'no, to whom we owe all our knowledge of *Tingiostachya*, writes: "The large tetralocular synangium of the present example"—numerous similar examples were found—"appears almost sessile on the subtending bract, but it may be more natural to expect the actual existence of a very short petiole (*sic*), although almost invisible, from the fact that a single circular attach-
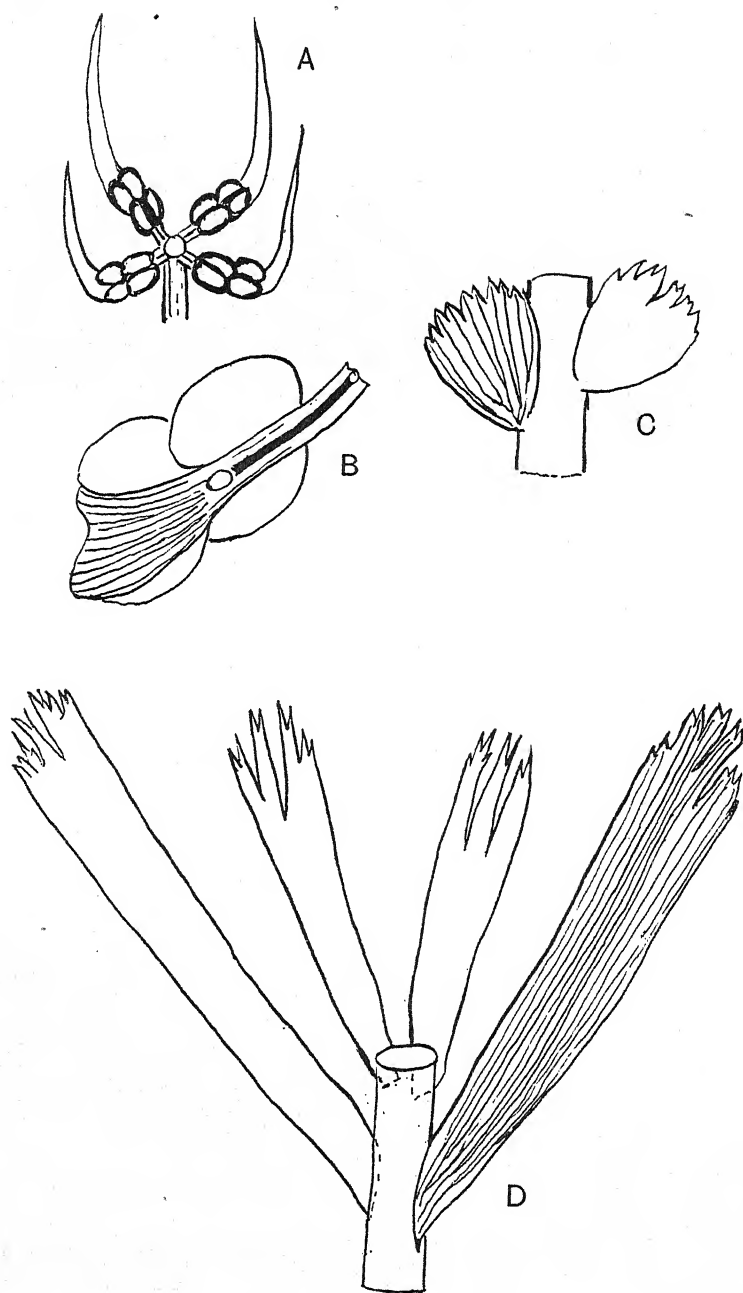
Fig. 2.

ment of the synangium is clearly recognisable, not only on the upper, but also on the lower surface of the bract; and one thick projection indicating the vascular supply from the rachis (*sic*) dies away quite abruptly at the very region of the synangium attachment on the bract, which is doubtless not to be interpreted as the midrib or as the petiole of the sporophyll itself" ((12), pp. 130–1). Thus he seems to think (1) that the projection running from the axis of the cone (which he in one place terms a rachis) to the attachment of the synangium probably contains the vascular supply of the latter; (2) that this projection may, perhaps, be regarded as a stalk or pedicel (called a petiole by him) of the synangium; and (3) that it is possible that, terminating this projection, there is a very short, hardly distinguishable free stalk bearing the synangium. As the vegetative leaves of *Tingia* have, like those of *Noeggerathia* and *Plagiozamites*, no midrib the first conclusion seems justified, while the two others do not seem to be at all improbable.

Professor Kon'no discusses very fully the affinities of *Tingia* ((12), pp. 130–7), and his discussion is, so far, the only one that takes the fructification into account. While rejecting any close affinity to the Psilophytales—a conclusion which certainly seems justified—he compares *Tingia* to the Sphenophyllales, Lycopodiales and Psilotaceae. Curiously enough, he makes no allusion whatever to *Noeggerathia* and *Plagiozamites*, although there are repeated refeiences in his text to Dr Halle's paper in which the affinity of *Tingia* to these genera is suggested and in which a reference is made to the fructification described by Sterzel for *Noeggerathia zamitoides* ((5), p. 227 and pp. 238–9). Professor Kon'no concludes that the recent Psilotaceae are, of all known plants, those to which *Tingia* comes nearest ((12), p. 135). He considers that *Tingia*, even in its fertile branches, was too different from the Sphenophylleae for comparison; and that it "showed no closer relationship to any fertile branches of the Spheno-

Fig. 2. *Tingia*. A. Restoration of a whorl of four sporophylls, showing sporangia on the upper surface. Magnified. B. A single sporophyll in back view, showing Kon'no's "thick projection" (blackened) and his "circular attachment," showing through on the lower side of the sporophyll. Considerably enlarged. C. Two of the larger leaves from the lower part of a shoot of *Tingia*. Natural size. D. Reconstruction of part of a shoot of *Tingia*, showing the four rows of leaves. The shoot was plagiotropic and the larger leaves are believed to have been on the upper side. Natural size. A and B after Kon'no ((12), p. 129, Text-fig. 4), modified. C and D, reconstructions from Halle's figures and descriptions ((5), pp. 230–9, Pls. 61–3).

phylleae than that existing between the recent Psilotaceae and the latter." On the other hand, he believes that *Tingia* "showed much closer resemblance to the Lycopodiaceae and Lycopoditeae than the recent Psilotaceae show to the latter" ((12), p. 137).

It seems to me that, taking into consideration the similarities between *Tingia* and *Noeggerathia*, the nearest affinity of both (and presumably also of *Plagiozamites*) is with the Sphenophyllales. The numerous sporangia on the upper surface of the bract, the relatively large simple leaves, into which several bundles obviously passed, the dichotomy of their veins and the way the ultimate branches of the latter terminate in the teeth of the margin of the leaf are all points of similarity to the Sphenophyllales, especially to those with undivided wedge-shaped leaves, a type of leaf that is curiously similar to the sporophylls of *Noeggerathia foliosa*.

Such is not Professor Kon'no's view. "The terminal position of the strobilus," he writes, "is quite rare in the fertile branch of the Sphenophyllales, although in the recent Equisetales it is sometimes observable; the dichotomous branching of the fertile branch is much rarer in the family, although so far as the author knows, one solitary example of such a case is known in *Schizoneura*" (generally classed with the Equisetales) "in *Tingiostachya*, on the contrary, the fertile branch shows splendid dichotomous branching....Moreover the cone axis is never articulate; the grouping of each of the four sporophylls simulating a whorl may not be interpreted as the radial arrangement of them as in the cases of the Sphenophylleae, but as their opposite attachment on the axis in four vertical rows...; none of the four sporophylls are (*sic*) ever webbed at the base and their lamina (? laminae) never dichotomize; the synangium is almost sessile, never showing any trace of such a long petiole or peltate lamina under which sporangia hang, as generally in the polylocular Sporangiophores of the Sphenophylleae, although in *Sphenophyllum majus* and in *S. trichomatosum* sessile sporangia are attached to the upper portion" —upper surface appears to be meant—"of the bract, each of the four in a group or singly, respectively" ((12), pp. 136–7).

It may be pointed out that the cones of *Sphenophyllum* were usually borne at the end of branches, though in some species, e.g. in *S. majus* Bronn there were no distinct cones and the fertile leaves were disposed in whorls, or zones of whorls, and were but little different from the vegetative ones ((25), p. 100). As the cones of *Tingia* have only been found detached, it is not certain whether they were borne terminally on forking main stems, or, as seems more likely,

terminating lateral branches, as usual in *Sphenophyllum*. Admittedly, the fertile branches of the Sphenophyllales do not seem to have been dichotomous. But neither were those of *Noeggerathia*, a genus resembling *Tingia* and usually placed in the same group ((5), p. 238; (19), p. 114). Moreover, in the Protoarticulatae, usually regarded as allied to but more primitive than the Articulatae and included by Drs Hirmer and Němejc with them in the Articulatales (7, 19), the fertile branches are repeatedly irregularly branched. It is true that the two genera of this group, *Calamophyton* and *Hyenia*, are probably closer to the Equisetales than to the Sphenophyllales, since their sporangiophores are borne on the axis and not on the leaf.

Is there any trace in the Noeggerathiales of the sporangiophore, that organ so characteristic of the Sphenophyllales? In the case of *Noeggerathia* nearly all who have described the fructification have spoken of the sporangia as sessile on the sporophyll (35, 29, 18). Geinitz, the first to describe it, alone talks of the sporangia—his "Früchte," for he regarded his detached specimen as a Gymnospermous cone— as being prolonged at their lower end into thin stalks ((2), pp. 391–2). Too much weight must not be attached to his unsupported statement, since he appears to have had but a single specimen before him. The marks in his figure ((2), Pl. III, fig. 1), where the sporangia have fallen off, are more or less circular and like stalks in transverse section, though hardly more so than the corresponding marks in some of Dr Němejc's figures (e.g. (18), Pl. I, fig. 2). Still, apart from Geinitz's statement, doubts as to the sporangia of *Noeggerathia* being completely sessile are permissible. Their falling off so very freely rather suggests the presence of a stalk. Then, again, Weiss, in 1879, spoke of the scars left on impressions by the sporangia as standing at the end of short linear projections on the upper surface of the leaf (sporophyll). He calls these "Kurze gestreckte Polster" ((35), p. 112) and points out that in the original specimens there must have been rounded impressions, each standing at the end of a small furrow ("...so sind es also eingesenkte Gruben oder Furchen mit rundlichen Narben die am Ende je einer kleinen Rinne stehen" (35), p. 112). Stur, who made a detailed study of *Noeggerathia*, notes six years later a curious point, namely that in the impressions left by the sporangia on the sporophylls, he frequently observed a split ("Riss"). This split he interpreted as representing the vertical line of dehiscence of the sporangium (31). It is hardly likely that the dehiscence of the sporangia of *Noeggerathia* would be on the side of the sporangium that was pressed against the sporophyll. A more probable suggestion seems to be that

this split, running vertically along the sporangia on the side towards the sporophyll, represents the distal end of a stalk (or sporangiophore), which would then be attached to the upper end of the sporangium, much as in *Sphenophyllum Dawsoni* Will. (cf. Dr Scott's figures (25), Fig. 44 *a*, p. 90 and Fig. 47, p. 95). Weiss' furrows, running to the scars left by the sporangia, would represent the proximal parts, adnate to and somewhat sunk in the tissue of the sporophyll, of these stalks. Professor Kon'no's "thick bundle," running to the insertion of the synangium of *Tingia*, is described as projecting also on the lower surface of the proximal part of the sporophyll, so that it, too, was partly sunk in the tissue of that organ. This "thick bundle" could also be regarded as composed of the fused pedicels of the sporangia composing the synangium. In any case it would be possible to look upon the "thick bundle" as the proximal part of a pluri-sporangiate sporangiophore, comparable to that of *Bowmanites Römeri* Solms (cf. (25), pp. 96–9), the distal part of which would be represented by the short synangial stalk, of which Professor Kon'no suspects the "actual existence."

It would be rash, in the face of the assertions to the contrary of those who have studied the cones more recently (29, 18), to affirm that the sporangia are not sessile. And yet, so experienced an observer as the late Dr Kidston was deceived into believing that the four sporangia borne in a group on the upper surface of the sporophyll of *Sphenophyllum majus* Bronn were sessile, whereas it has recently been shown that there was a short common stalk or sporangiophore to them ((6), p. 42). But, though *S. majus* has, since the appearance of Professor Kon'no's paper, been shown to possess a sporangiophore, there remains the second species mentioned by him as having sessile sporangia, *S. trichomatosum* Stur, in which a single, and so far as we know, a sessile sporangium is borne on each bract ((7), p. 360). So that even if the sporangia of *Noeggerathia* were truly sessile on the sporophyll, we should not be precluded from comparing these plants to the Sphenophyllales, though the presence of a sporangiophore would be much more favourable to the comparison. Dr Němejc's figure of a longitudinal view of a cone of *Noeggerathia foliosa* ((18), Pl. I, fig. 5) and Dr Scott's reconstruction of a longitudinal section through a cone of *Sphenophyllum Dawsoni* Will. ((25), Fig. 44 *a*, p. 90) are in many ways strikingly and curiously alike. There is the same arrangement of relatively numerous sporangia in transverse and longitudinal rows on the upper surface of leafy sporophylls. In no other group do we find anything really comparable to this. Because the Noeggerathiae

are not articulate forms, are we to refuse to see in these similarities and in the possession of simple pseudo-macrophyllous leaves with numerous forking veins indications of a real relationship?

I do not think so. Though the most recent work seems to show that even in the oldest of the Articulatales, in the Protoarticulatae, the leaves were disposed in regular whorls ((15), p. 326), the axes of these plants were irregularly dichotomously branched; and Drs Kräusel and Weyland look upon *Hyenia* as not definitely articulate (15). Again, the fact that no Sphenophyllales have been recognised below the Upper Devonian and very few below the Carboniferous might equally well be rendered by the generalisation that before this time their forebears had acquired neither the articulation nor the verticillate phyllotaxy that we are accustomed to regard as a criterion for inclusion in the phylum.

If the similarities between Noeggerathiales and Sphenophyllales be taken as indications of affinity we may suppose that the, on the whole younger, group of the Noeggerathiales has been derived through loss of verticillation of the leaves and partial (*Tingia*) or complete (? *Noeggerathia*) obsolescence of the sporangiophores (or pedicels of the sporangia) from forms which, if we knew them, we might class with the Sphenophyllales. The verticillation of the sporophylls of *Tingia*, if not secondary, may be used to support this view; and so may the fact that the leaves of the Noeggerathiales are closer to the undivided wedge-shaped form of *Sphenophyllum* leaf than to the narrow dichotomously forked leaves of the oldest known species, *S. subtenerrinum* Nath. from the Upper Devonian ((7), p. 361), a form of leaf usually regarded, since Lignier's work on the group (16, 17), as primitive. If the Noeggerathiales were derived from a common ancestor with the Sphenophyllales rather than from the latter group in the widest sense, we should expect their leaves to resemble the more primitive type of leaf found in the sister phylum. As both forms of leaf occur on the same individual, this point may not be of great importance. On the other hand, it may well be that the similarities between the two phyla indicate no closer affinity than a common origin and that the Noeggerathiales were derived from forms, unknown to us, forerunners of the Sphenophyllales, that had not yet acquired whorled leaves. But, whichever view we take, we must, it seems to me, recognise that the nearest affinity of the Noeggerathiales is with the Sphenophyllales. And this conclusion raises a further question, which it may be useful to state, even if we cannot answer it. Are we to discard any ideas of an affinity, closer than that hypo-

thecated for all Pteridophyta, between the Noeggerathiales, especially *Tingia*, and the Psilotales?

Our view of the affinities of the Psilotaceae will probably depend chiefly on how we interpret their sporangiferous organs and on whether or not we consider them as primitively microphyllous. If we look upon their fertile parts as branches, rather than as fertile leaves or sporophylls, we shall reject the validity of the comparison between them and *Tingia* and shall incline to regard the Psilophytales as the plants known to us that show most similarity to them. There is much to be said for this view, supported as it is by the dichotomous branching and complete rootlessness, even in the embryo, of the Psilotaceae ((8), p. 394). The affinity between the Psilophytales and Psilotaceae has been upheld by Drs Kidston and Lang ((11), pp. 839–43), by Professor Bower (1), by Dr Sahni (21, 22), and to some extent by Dr Zimmermann, who regards the Psilotaceae as derived from the Psilophytales, in the widest sense, but believes that this is also true of the other phyla of Vascular Cryptogams, and emphasises the absence of any intermediate forms between the two groups ((36), p. 125). Dr Scott, however, while regarding the affinities of the Psilotales as quite uncertain, looks upon them as more probably aberrant Lycopods. He thinks that they may have retained some primitive characters, also found in the Psilophytales, but rejects the idea of a near affinity between the two groups, as well as the idea, previously entertained by him ((24), p. 616) of an affinity between the Psilotales and Sphenophylls. He particularly stresses the constancy of the whorled phyllotaxis in the Sphenopsida, stating that it should probably be accepted as an essential character of the group ((26), p. 398). When Dr Scott introduced the conception of the sporangiophore into the interpretation of the fertile parts of the Psilotaceae, he, having at that time a belief in an affinity with the Sphenophyllales, regarded the synangium, with its stalk, as a sporangiophore, a ventral lobe of a compound sporophyll of which the two small laminae represented dorsal lobes. Dr Sahni, however, applies the term sporangiophore to the whole structure. He regards the sporangiophore as a branch system, bearing, in a normal plant of *Tmesipteris*, two leaves, strictly homologous with vegetative leaves, and a cluster of sporangia, interpreted as terminal and united into a synangium ((22), p. 162). It is hard to accept this view, for each such leafy lobe associated with the synangium is about half the size of the vegetative leaves of the same part of the axis, and the two lobes, together with the synangium, occupy the position on the axis

of a single vegetative leaf. This is particularly clear when the fertile organs occur, as they sometimes do, singly between the ordinary leaves (cf. (3), p. 1103 and Fig. 1086; (32), p. 65, Fig. II). Dr Sahni himself frankly points out that neither in his serial sections containing intermixed traces of sporangiophores and leaves, nor in his separate series of leaf and sporangiophore-traces could he find any constant difference, such as it had previously been suggested occurred ((22), p. 155) in structure or mode of departure of the traces of leaves and sporangiophores. He quotes with approval Mrs Thoday's (Miss Sykes') remark that an abnormality described by her (33), in which a branched axis bore several leaf-like lobes and sporangial clusters in different planes, could not readily be taken for a modified leaf, but that it might well be described as a branched leaf-bearing sporangiferous shoot ((21), p. 185). There seems, however, nothing inconsistent with the nature of a sporophyll in this branching and bearing of sporangia in more than one plane. Both features are characteristic of the sporophylls of the Sphenophyllales, e.g. of those of *Cheirostrobus*. And, while it is doubtful how far abnormalities should be used as a basis of morphology, there seem to be quite as many of them supporting the foliar view of the fertile organs: e.g. the occasional forking of the vegetative leaves in *Psilotum* ((30), p. 380); the apparent incipient forking of vegetative leaves in *Tmesipteris* ((8), p. 417, Figs. 93, 94 and 95 *a*) and the repeated occurrence, in Australia, of unbranched leaf-like sporophylls in the latter genus ((3), p. 1102).

On all these grounds the fertile organs of the Psilotaceae seem to be best regarded as compound sporophylls. Ultimately, both compound leaves and branches have presumably been evolved from branching thalloid axes, so that it is possible to regard the fertile organs of the Psilotaceae as *ultimately* homologous with both leaves and branches ((36), p. 125). But, as Drs Kidston and Lang have pointed out, the sporangiophores of the sporangiophoric Pteridophyta and, in somewhat different fashion, the fertile appendages of, *inter alia*, the Psilotaceae may be regarded as the last persisting remains of the leafless branch systems of the Rhyniaceae ((11), p. 850); and the point here argued is that in the Psilotaceae these last persistent leafless branches (or sporangiophores) persist as such only in the ultimate ventrally situated branch of the fertile leaf, the synangium, with its vascularised stalk.

Again, are we to look upon the Psilotaceae as primitively microphyllous? If we do this, we shall probably incline to Dr Scott's tentative suggestion that they are aberrant Lycopods. Or are we to regard

their leaves as reduced from larger ones, with several vascular bundles, more resembling those of the Noeggerathiales? The difficulty is that the two surviving genera are so much modified, *Psilotum* obviously by reduction and *Tmesipteris* by xerophily, exemplified in the vertical orientation and leathery lamina of the leaf ((3), p. 1021). The forking of the sporophylls, which in *Tmesipteris* is said to be not infrequently repeated when nutritive conditions are favourable ((34), p. 354), and the fact that near the apex of the shoot of the holophytic *Tm. Vieillardi* Dangeard "the stele appears to be composed entirely of decurrent leaf-traces and the axis of coalescent leaf-bases" ((22), p. 153) support the view that the leaves were neither primitively unforked nor small compared to the axis. The genus *Sphenophyllum* itself shows much variation in the size and form of the leaf. It includes *S. longifolium* Germar, with entire or twice forked leaves, up to 4 cm. long, *S. majus* Bronn, in which the leaves are either slightly lobed or forked up to four times ((7), p. 362), as well as *S. Wingfieldianum* Hemingway, in which the leaves are bifid with a forking bundle, or entire, with an unbranched vascular strand (6). Thus, it seems not improbable that the leaves of the Psilotaceae were not primitively truly microphyllous. If this was so, the chief argument in favour of an affinity with the Lycopods drops out and we are left with the vegetative characters (rootlessness, dichotomous branching) suggesting a certain affinity with the Psilophytales, while the character of the sporangiferous parts points rather to an affinity with the Noeggerathiales, particularly with *Tingia*.

What classification best fits these bewildering facts? We may perhaps accept Dr Němejc's seven groups, viz. the Psilophytales, Lycopodiales, Psilotales, Noeggerathiales, Articulatales, Cladoxylales and Filicales, as including within them the better understood types of Pteridophyta. But is it not possible that, while regarding the Psilophytales as the most primitive group, nearest the prototypes of the other main groups, we should restore the threefold grouping of the other vascular cryptogams into Pteropsida, Sphenopsida and Lycopsida? The Pteropsida would presumably include the Cladoxylales and Filicales. The Sphenopsida would not be confined to the Articulatales, but would include, in the Noeggerathiales, nonarticulate forms in which the phyllotaxy was not verticillate. Should the Sphenopsida also include the Psilotales, or should these be placed next to the Psilophytales, or next to the Lycopodiales, in the Lycopsida? Perhaps the position of the Psilotales is best indicated by suggesting that their least distant affinities may be with the Spheno-

psida, especially with the non-verticillate Noeggerathiales, but that they have retained certain primitive characters (rootlessness, dichotomous branching) found also in the Psilophytales.

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FLORAL ANATOMY OF *RIVINA HUMILIS* L., AND THE THEORY OF CARPEL POLYMORPHISM

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(With 6 figures in the text)

S OME time ago, in the pages of this journal, Miss Saunders described the anatomy of flowers of some members of the family Phytolaccaceae(1) and from that derived various conclusions, one of which was that in the genus *Rivina*, the gynaecium is composed not of a single carpel, as had been believed so far, but of two carpels, one anterior, valve, sterile and possessing a style and another posterior, solid, fertile and without a style. Going over her description of the plant and figures, we found that the anatomy of the gynaecium in *Rivina* is very similar to that found in the family Nyctaginaceae on which we had been working for some time and in which we had evidence for believing that there is only a single carpel. It was unfortunately not possible to obtain proper material of more than one species, namely, *Rivina humilis* L. The study of this alone, however, confirmed our first suspicions as to the validity of Miss Saunders' conclusions and revealed also some differences from her observations.

The material of *Rivina humilis* was taken from the botanic garden of the Benares Hindu University and was fixed in a mixture of formalin, acetic acid and 60 per cent. alcohol. The observations are based on serial microstome sections of flowers just before opening. The accompanying figures are all camera lucida drawings.

OBSERVATIONS

The pedicel just below the receptacle shows a complete ring of vascular tissue (Fig. 1) having xylem on the inside and phloem on the outside. This ring enlarges slightly as it reaches the receptacle and here it becomes also somewhat rectangular, conforming more or less to the shape of the receptacle itself. Both these features are due to the presence of four perianth leaves in a flower. The perianth traces are, to begin with, only eight in number (Fig. 2). Four are given off

from the sides of the rectangular stele of the thalamus and these pass out undivided to the respective perianth leaves on the four sides and form their midribs. The other four traces are given off from the four corners of the central cylinder. Each of these divides at its very base into two equal branches which pass to adjacent perianth leaves and form their laterals. In this manner every perianth leaf gets three main vascular strands which may divide a few times (especially the laterals), or may not (generally the midrib bundles), but ultimately all reach the apex and there they fade away. Both the midrib traces and lateral traces of the perianth leaves cause gaps in the stele of the thalamus on their departure, but the gaps caused by the midrib traces are more prominent and close at a higher level than those caused by the lateral traces, which are very narrow and soon close up. Miss Saunders does not describe the origin of the perianth traces. The four perianth leaves do not arise at the same level. The antero-posterior pair is the first to detach itself from the floral axis and the lateral pair of perianth leaves is detached at a slightly higher level than the former (Fig. 3).

After the departure of the perianth traces, the vascular tissue of the floral axis again forms a complete ring and now there are given off four traces for the stamens, one for each. These alternate in their position with the midrib traces and are situated just above the points of origin of the lateral traces of the perianth leaves (Fig. 3). The origin of the staminal traces causes no gaps in the stele of the floral axis. It may happen that sometimes the gaps caused by the departure of the median perianth traces may not have closed at the level of origin of the staminal traces, but absolutely no gap is seen in the stele at the points of origin of the latter, and the vascular tissue of the floral axis continues to form a more or less closed ring. Miss Saunders on the other hand figures the staminal traces as causing definite gaps in the stele and leaving behind four separate strands which she describes as the traces of the gynaecium. Even after an examination of serial sections of a number of flowers we have not been able to see such a condition. The four stamens are detached from the floral axis almost simultaneously.

Immediately after the departure of the staminal traces, and just as these reach the base of their respective stamens, the stele of the floral axis breaks up into three strands, which to begin with are nearly of an equal size (Fig. 4). Higher up one of these becomes some-what smaller, while the other two unite to form one large bundle. In this manner a cross-section of the base of the ovary shows one large

and one small bundle (Fig. 5). The large bundle goes directly to supply the single basal ovule, while the smaller bundle passes into the ovary wall on the anterior side (Fig. 6). It gives a few branches during its course towards the style. A stylar canal (marked *st.* in Fig. 6)
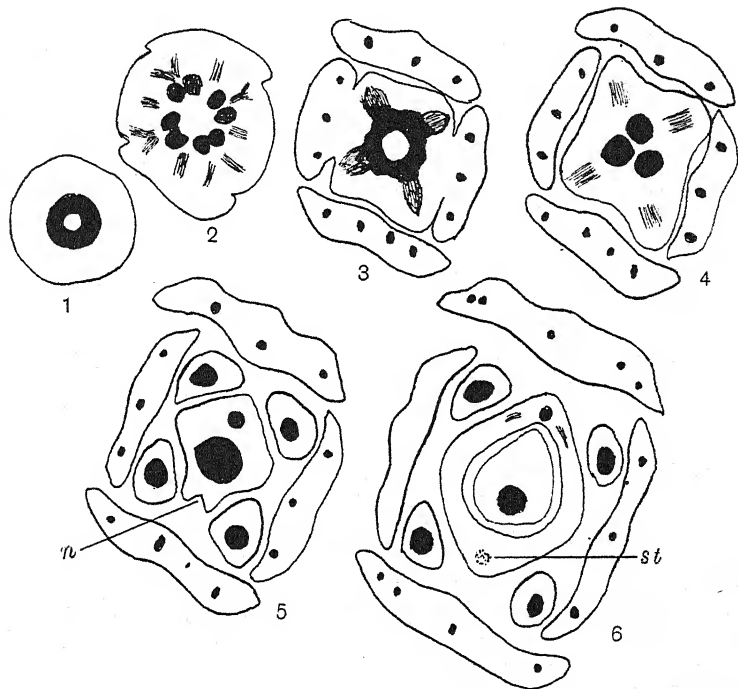
Figs. 1–6. *Rivina humilis.* Figs. 1–6 are cross-sections of a flower at various levels from below upwards. Fig. 1 shows the structure of the pedicel; Fig. 2, the origin of the perianth traces; Fig. 3, the separation of the perianth leaves and the origin of the staminal traces; Fig. 4, the three traces of the gynaecium; Fig. 5 the lateral notch (*n*) in the gynaecium; Fig. 6 shows the passing of the dorsal bundle into the ovary wall and of the ventral bundle into the ovule, and a stylar canal (*st*) on the ventral side in the ovary wall. Vascular tissue in Figs. 1–6 is represented in black when cut transversely, by lines when cut lengthwise. Magnification, × 48.

makes its appearance in the ovary wall on the side opposite the vascular strand, at some distance from the base, and both this and the vascular bundle ultimately pass into the style and end in the stigma. Miss Saunders in her account says that there are four traces for the gynaecium, but we have always found only three. This is of importance as it goes against her conclusions.

Another point which she does not mention but which appears to be of great importance to us is that on the posterior side of the ovary, i.e. on the side opposite to the one which receives the small bundle, there is a marked notch at the base. This is shown in Fig. 5. This notch is found just at the base of the ovary, at about the level at which the loculus of the ovary makes its appearance. It is not so long as it is in some other plants, but even so its presence is quite clear and no doubt is left about its significance when comparison is made with the related family Nyctaginaceae. In that family we have found that in species of *Boerhaavia* the carpel remains open along this line throughout its life even in the fruit; in *Mirabilis* there is a clear line at this place showing the fusion of the margins of the original carpellary leaf, while in *Bougainvillea* there is a notch at the same place. From this series it is clear that the notch also represents the line of fusion of the margins of an originally open carpel.

Discussion

The structure of the gynaecium then in *Rivina* is this. There are three traces, and this is generally believed to be the primary number in the floral leaves of flowering plants. One of these passes into the wall of the ovary, while the other two jointly supply the basal ovule. Secondly, there is a distinct notch in the ovary at its base on the posterior side, which from comparison with the family Nyctaginaceae is seen to represent the line along which the fusion of the margins of the originally open carpel has occurred. The bundle traversing the wall of the ovary is situated opposite to this notch. The simple conclusion from this evidence is that there is only a single carpel in the gynaecium of *Rivina*. The bundle supplying the ovary wall has to be interpreted as the midrib bundle of the carpel or the so-called dorsal bundle, and the two bundles supplying the ovule are to be regarded as the marginal bundles of a three-traced carpel. The latter have united and do not extend into the ovary wall, as there is only a single basal ovule. The position of the notch on the side of the ovary opposite to that of the dorsal bundle fully supports such a view. If there had been four traces for the gynaecium as Miss Saunders describes then there would have been some support for concluding that there are two carpels in *Rivina*. The present investigation shows that there are only three traces and there is absolutely no ground for her view. Eames (2) has already pointed out the inconsistency in Miss Saunders' conclusions in regarding the carpels of *Phytolacca, Ercilla,*

etc., as monomorphic and those of *Rivina* as polymorphic when all have the same vascular anatomy. He would call them all monomorphic and the present observations support his conclusions.

The monocarpellary view of the gynaecium of *Rivina* is also supported by a study of its development. A study undertaken by one of us shows that the carpel at first develops only on one side of the terminal ovule—on the side away from the axis of the inflorescence—and only later on encloses the other side. Such a development of the gynaecium is quite similar to the development of a single carpel of *Ranunculus*(3) or that of *Alchemilla*(4).

Summary

The anatomy of flowers of *Rivina humilis* is described. Two important differences are found from the account given by Saunders. First, the staminal traces do not cause any gaps in the stele of the floral axis on their departure and secondly, there are only three traces for the gynaecium.

In addition to this it has been found that there is a distinct notch on the posterior side of the gynaecium in *Rivina* which from comparison with other plants is seen to represent the line of fusion of the margins of an originally open carpel.

From these observations it is concluded that there is only a single carpel in this genus.

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STUDIES OF THE PHYSIOLOGICAL IMPORTANCE OF THE MINERAL ELEMENTS IN PLANTS

V. THE DISTRIBUTION OF DIASTASE, INVERTASE AND CATALASE IN NORMAL AND POTASSIUM-STARVED BEAN PLANTS

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(With 3 figures in the text)

INTRODUCTION

IT is well known that one of the principal symptoms of potassium starvation in plants is a profound disturbance of the carbohydrate metabolism. Some workers (Maskell, 1927; Nightingale, 1930; James, 1930) have recorded marked decrease of starch and protein formation, while others give results of a very complex nature (Bobrownicka-Odrzywolska, 1926; Janssen and Bartholemew, 1930). A promising method of achieving a better understanding of these changes seemed to lie in a study of the enzyme systems involved. The data already recorded (Doby and Hibbard, 1927; Hartt, 1929) are, however, very incomplete and contradictory. As a first step towards a solution, a comparison of the enzymatic activity of various parts of normal and potassium-starved bean plants was undertaken, the results of which are given in this paper. The enzymes chosen for the work on account of their importance and suitability for manipulation were diastase, invertase and catalase, and as their behaviours differ to some extent, each is treated separately below.

METHODS

Cultural methods

A pure line of broad bean (Sutton's Exhibition Longpod) was used for the experimental work described, the seeds being graded by weighing to secure increased uniformity. The beans were sterilised in alcohol and germinated in sand, and when the roots were 2 in. long the seedlings were transferred to normal and potassium-deficient

culture solutions (according to formulae in James (1933)). The usual precautions were taken and the solutions renewed every three weeks.

Starved and normal beans began to differentiate after about four weeks in culture, the potassium-starved plants being shorter than the normal plants and showing an abundance of freak leaves. The marginal scorch characteristic of potash starvation appeared on the lower leaves, which shrivelled but remained attached to the plant, and if flowering occurred, the starved plants were always in advance of the controls.

When the eighth or ninth leaf was expanding the plants were used for experimental purposes. In summer the controls showed all leaves in a healthy condition, but the starved individuals had only four or five green leaves, the rest having withered. In winter both starved and normal plants were in poor condition; there was very little difference between the two types of plant, and the lower leaves of both were shrivelled.

The determinations were confined to the leaves, as it was impossible to carry out any additional estimations, e.g. of the petiole or internodes, without too long a time interval elapsing between the first and last sampling. Especially was this so when the enzyme was extracted from fresh material.

In each set of cultures the leaves were removed successively from the apex downwards, and numbered in a similar order. The first leaf to be investigated had developed beyond the bud stage although the leaflets were still folded, while the second leaf had already reached maturity. The leaves from corresponding nodes were treated as one sample.

Experimental methods

Preparation of enzyme.

Diastase. The leaves were thoroughly dried at 30–33° C. for four days, and the material was ground to a powder and extracted with distilled water and toluol. The extract was filtered through muslin, and the residue washed twice and the washings added to the extract, which was then made up to a known volume.

Invertase and catalase. An extract was obtained from the fresh leaf material. This was thoroughly ground with washed sand, extracted with water and toluol, and filtered through muslin, and the solid residue washed free from traces of enzyme. Preliminary experiments determined the length of time necessary for complete extraction of the enzymes, thus:

Extraction time for diastase was 4 hours.
    ,,   ,,   invertase was 3 hours.
    ,,   ,,   catalase was 10 min.

Further purification of the enzyme was considered to be unnecessary and undesirable, since it was the total enzyme content that was being considered, and precipitation would introduce additional sources of error.

Determination of diastatic activity of extract.

Diastatic activity was determined by the rate of formation of reducing sugar in a starch-enzyme digest at a temperature of 30° C. The digest was prepared as follows:

> 25 c.c. 0·5 per cent. Lintner's soluble starch solution.
> 2 c.c. enzyme extract.
> Hydrochloric acid to give pH 5.
> Toluol.

5 c.c. portions of the digest were removed by means of a standardised pipette immediately after mixing the digest and after one hour at 30° C., and the amount of reducing sugar present was estimated by the method of Hagedorn and Jensen (1923) as modified by Hanes (1929). An action time of one hour was taken in order to be sure that the activity was determined on the linear portion of the progress curve.

Estimation of the starch content of leaves.

It was necessary to devise some quantitative method of starch estimation which could be carried out fairly rapidly, and the following procedure was adopted and found to give satisfactory results. A starch scale with a range of 0–8 was made up in corked comparator tubes using ten drops of iodine and 10 c.c. of starch solution in each tube. The concentration of starch solutions was noted and the scale could therefore be made up repeatedly without undue error. The leaves to be tested were decolorised by alcohol in the usual way, and as many discs as possible (diameter 1 cm.) were cut from them. The discs were thoroughly ground in sets of ten with 10 c.c. of distilled water until a uniform opaque suspension was obtained. To this was added ten drops of iodine solution, and the suspension was compared colorimetrically with the starch scale by transmitted light. A suspension of gamboge was placed behind the starch scale to compensate for the yellow colour of the solid material in the leaf suspension.

Determination of invertase activity.

Invertase activity was estimated by the formation of reducing sugars in a sucrose digest of the following composition:

> 50 c.c. 2 per cent. sucrose solution.
> 2 c.c. enzyme solution.
> Hydrochloric acid to give pH 6.
> Toluol.

The Hagedorn-Jensen titration was used, and the method was therefore essentially similar to that used for diastase.

Determination of catalase activity.

A modification of the iodometric method (Waksman and Davison, 1926) was used for the measurement of the catalase activity. The enzyme is introduced into a hydrogen peroxide digest, and after a convenient period of incubation the residual hydrogen peroxide is estimated by the liberation of an equivalent quantity of iodine from a solution of potassium iodide. The iodine is then titrated with standard thiosulphate. The concentrations given by Waksman and Davison proved unsuitable for bean catalase and as a result of preliminary experiments the following standard procedure was adopted:

Digests of 5 c.c. hydrogen peroxide solution (1 volume available O_2),

> 2 c.c. extract,
> pH 7, due to natural buffers.

were incubated at 30° C. for 15 min. To each digest was then added

> 10 c.c. of 10 per cent. sulphuric acid,
> 10 c.c. of 5 per cent. potassium iodide.

After allowing the mixture to stand for one hour, the iodine liberated was titrated with standard $N/20$ sodium thiosulphate solution using as indicator fifteen drops of 0·5 per cent. starch solution.

Determination of amino-acid content of extract.

The amino-acid content was determined by Sörensen's formol titration (Lieb, 1931) as modified by F. J. Richards (unpublished work). When a neutral formaldehyde solution is added to an amino-acid solution, the amino groups form a methylene group with the formaldehyde, so that it is possible to estimate the carboxyl groups titrimetrically.

The proteins and dark colouring matter were precipitated from the experimental solution by the addition of barium chloride in

alkaline solution, and a pale amber-coloured liquid was obtained on filtering. This was titrated with hydrochloric acid to an initial value of pH 8, using phenol red as indicator. 8 c.c. of 36 per cent. formaldehyde (containing the same proportion of phenol red, and previously titrated to the same pH with caustic soda) was added to each 10 c.c. sample of filtrate. The sample was then titrated back to pH 8 with a normal caustic soda solution, this being the required titration. The accuracy of the titration depends to a great extent upon the relative amounts of water and formaldehyde present—increase of water will tend to reverse the direction of the reaction, and complete estimation of the amino-acids will be impossible. It is an advantage, therefore, to use normal caustic soda for the titration to avoid dilution of the sample, and a microburette was employed for the measurement of the small quantities of caustic soda. A mercury plunger, controlled by a screw, forced liquid out of a fine capillary jet in minute droplets. The volume of liquid expelled was indicated by the position of the mercury in a calibrated tube. By working in this way at pH 8 and with a final formaldehyde concentration of 16 per cent. only about 95 per cent. of the amino acid is estimated. It was necessary, therefore, to construct a standardisation graph using pure amino acids. The relationship between caustic soda and amino acids is found to be a linear one, and a constant (0·07 c.c. $N/1$ caustic soda = 1 mg. nitrogen) was therefore used for the conversion of the alkali values.

EXPERIMENTAL RESULTS

Diastase

The investigation was carried out along three lines of approach:

(1) The diastatic action of an extract of leaf material was measured by the rate of formation of reducing sugars;

(2) The rate of loss of starch was determined in detached leaves kept in the dark;

(3) The rate of starch formation was investigated in detached leaves floated on sugar solution.

That is, hydrolysis by the enzyme after extraction, and hydrolysis and synthesis in the leaf were each examined. The breakdown of starch by the extracted enzyme as shown by an iodine method could not be examined on account of the difficulty of growing sufficient material in water-cultures.

The consideration of such a group of results is necessary in the case of diastatic action, since an enzyme system rather than a single

enzyme is involved. In fact the problem is further complicated by the possibility of the presence of two separate systems—activating hydrolysis and synthesis respectively. Thus Giessler (1928) finds that salt solutions increased the rate of loss of starch from detached *Drosera* leaves, but hindered the formation of starch in leaves floated on sugar solution.

(1) *Diastatic activity of leaf extract.*

Preliminary experiments were carried out, using winter-grown material, with only three green leaves on both normal and potassium-deficient plants. The results were slightly irregular, the most notable feature being the greater activity of the second leaves of the potassium-deficient plants as compared with those of the normal plants. Statistical analysis by the application of Fisher's "t" test (1932) to the results showed the average activity of the normal plants to be significantly higher than that of the potassium-starved plants.

For more detailed investigations, summer-grown plants were used, and here the results were regular, and the plants showed a normally healthy growth.

It seemed possible that the distribution of diastase might show some correlation with the amino-acid content of the leaves, since the "complement" of amylases is said to consist of the degradation products of proteins (Haldane, 1930). Estimations of the diastase and amino-acid content of the leaves were therefore carried out.

There was a distinct difference between the normal and the potassium-starved plants, in the number of their green leaves— normal plants having an average of 6·7, and potassium-deficient plants an average of 4·5. The leaves of each node were divided into four equivalent samples as they were removed from the plants, and the fresh weight and area determined for each sample. To obtain the areas, the leaves were placed between two glass plates supported above an electric lamp. The outline of the leaf was then clearly defined on a sheet of paper placed above the glass, and the leaflets were drawn. The shapes were then cut out, dried and weighed. The area was calculated according to the average weight of 1 sq. cm. of the paper used. This method of area determination was found to be as accurate as the planimeter method, and to be more rapid in execution.

Two of the equivalent samples were dried at 30° C. and subsequently tested for diastatic activity; the other two were plunged into boiling water to eliminate autolysis by rapid killing, and then dried at 85° C. and tested for amino acids.

In determining the diastatic activity, three digests were set up for each set of leaves, and three estimations were made for each digest. Thus each result was obtained as the average of eighteen experimental values.

In order to obtain a sufficiently large sample for amino-acid estimation, the two sets of material from each node were mixed and treated as one sample. Even so, the low amino-acid content of the leaves and the relatively large volume of solution needed for each estimation, made it impossible to obtain more than one value for the leaves from each node.

The results are given in Table I, and are expressed on several bases of comparison—1 gm. fresh weight, 1 gm. dry weight, per leaflet, and

TABLE I

Distribution of diastase and amino acid

Leaf	Cul-ture	Diastase per				Amino-acid per			
		Leaflet	1 gm. fresh wt.	1 gm. dry wt.	100 sq. cm.	Leaflet	1 gm. fresh wt.	1 gm. dry wt.	100 sq. cm.
Top	K	1·573	31·20	163·0	67·9	0·0120	0·2217	1·281	0·4874
	K—	1·057	21·32	112·55	42·6	0·006	0·1172	0·655	0·2501
2nd	K	5·470	39·82	236·9	77·8	0·0306	0·2326	1·339	0·4628
	K—	3·660	30·58	191·75	64·1	0·0243	0·1984	1·209	0·4032
3rd	K	7·366	32·62	228·35	66·9	0·0455	0·2001	1·359	0·4012
	K—	5·795	26·82	200·5	56·0	0·0390	0·1830	1·325	0·3672
4th	K	5·867	19·32	142·2	41·5	0·0388	0·1296	0·910	0·2804
	K—	4·415	15·59	117·6	32·4	0·0442	0·1481	1·100	0·3331
5th	K	4·497	10·47	87·27	24·7	0·0350	0·0842	0·6495	0·1988
	K—	2·547	6·708	53·48	15·3	0·0445	0·1125	0·9315	0·2616
6th	K	3·967	7·024	61·69	18·2	0·0383	0·0668	0·5716	0·1730
	K—	2·603	4·692	38·34	12·0	0·0725	0·1283	1·047	0·3158
7th	K	4·267	5·498	50·37	16·2	0·0748	0·0942	0·8318	0·2768
8th	K	2·536	3·491	35·35	11·4	0·0623	0·0871	0·8492	0·2932

per 100 sq. cm.—as the problem of finding a suitable method of comparison is a very real one. Other investigators have used dry weight alone as their unit, but it is not yet known how far such a basis is valid since the dry weight itself depends on a complex interplay of enzyme activities.

By whichever method the plants were compared it was found that lack of potassium depressed their diastatic activity (sugar production) in leaves of all ages (see Fig. 1 for comparison of equal areas). The amount of depression varied with the age of the leaf as shown by its position on the stem. The percentage depression was least in the third leaf and greater in those both younger and older. Analyses by
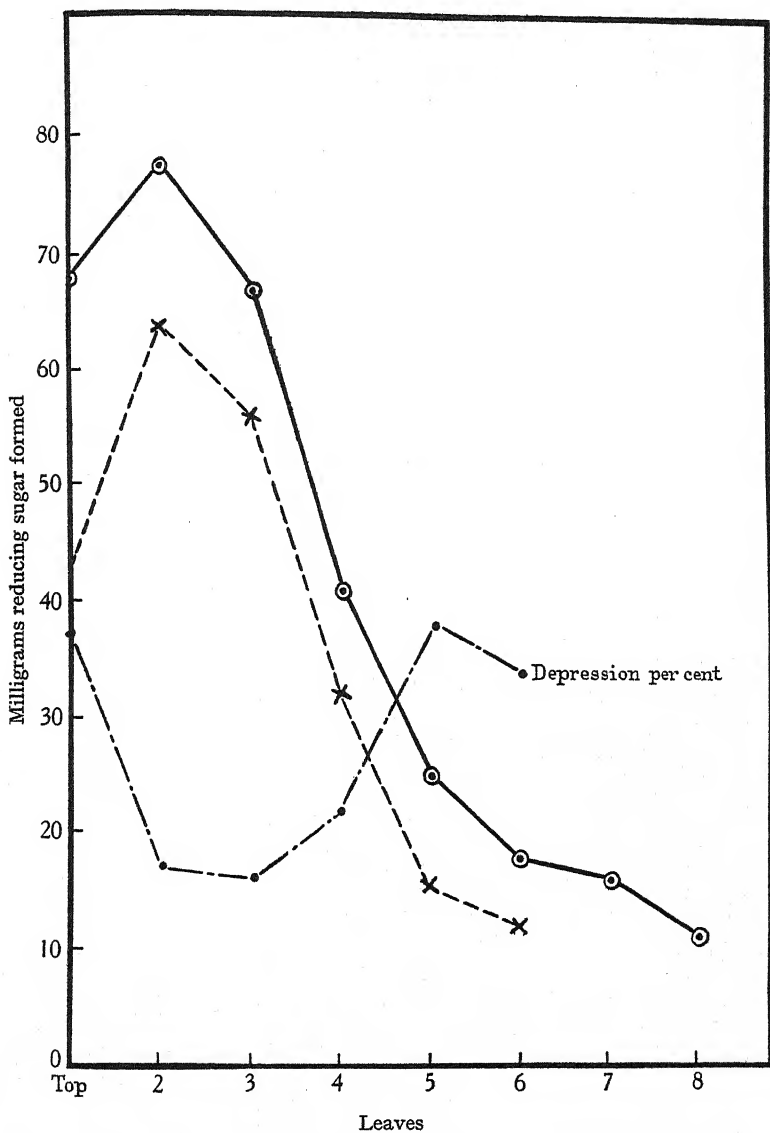
Fig. 1. Distribution of diastase in leaves, expressed as units of reducing sugar formed per 100 sq. cm. of leaf.

⊙— plants grown with complete culture solution.

×--- plants grown without potassium in the culture solution.

Mr F. B. Hora showed that the depression of actual potassium content of the leaves due to potassium starvation showed a minimum at the same leaf.

The distribution of diastase and amino acids down the stem of the normal plants showed some slight resemblance, but the effects of potassium starvation were different: starved plants showed a subnormal diastatic activity all down the plant whatever the method of comparison, but although the amino-acid content of the top leaves was subnormal, a definite cross-over was seen and the lower leaves of the starved plants had a supernormal amino-acid content. A determination of the amino-acid content of the actual extract after autolysis again failed to show any correlation. The effect of potassium on the enzyme cannot, therefore, be explained by a control of the amino-acid content.

It is interesting to note that in a series grown in October, the potassium-starved plants showed a higher diastatic activity in the second leaves, and similar results were obtained in the preliminary experiments on winter-grown material. This aberration may be important in explaining differences between the above results and those of other workers.

(2) *Rate of loss of starch in detached leaves.*

Sixty plants (thirty normal and thirty potassium starved) were left under a 1500 c.p. electric lamp for 24 hours to obtain a high starch content. The plants that were used were rather far advanced, however, each plant showing thirteen or fourteen leaves instead of the usual eight or nine and the starch content was low. The leaves were detached and placed in the dark in distilled water, samples being tested for starch immediately and after 2, 4, 6 and 18 hours. As many readings as possible (approximately twelve from each leaf at each time) were taken from each sample.

The results obtained are given in Tables IIA and B and were submitted to statistical analysis (see Table IIc) by Fisher's "z" method (Fisher, 1932). It was then found that the difference due to treatment, supplying or withholding potassium, was definitely significant (P considerably less than 0.05), and that there were also significant changes of rate with advancing time and according to the position of the leaf examined. The average rates, estimated per unit of starch present at the beginning of the period, were 0.3449, 0.5772, 0.5758, 0.2677 respectively and show that the rate lags at first and is not proportional to the total amount of starch present. The higher rate

of disappearance in the normal leaves compared with the starved is not therefore due merely to the greater amounts of starch that they possess initially, as might be supposed from a first examination of the figures.

TABLE IIA

Starch in detached leaves

Leaf	Culture	Immediately	After 2 hours	After 4 hours	After 6 hours	After 18 hours
Top	K	2·3	2·2	1·3	0·8	0
	K—	1·7	1·6	1·4	1·1	0·7
2nd	K	3·4	2·8	2·0	2·1	0·7
	K—	2·0	1·4	1·1	0·9	0·8
3rd	K	3·6	3·2	2·7	2·3	0·7
	K—	1·3	1·4	1·25	1·15	0·7

TABLE IIB

Rate of loss of starch per hour per unit of starch

Leaf	Culture	0–2 hours	2–4 hours	4–6 hours	6–18 hours
Top	K	0·0217	0·2045	0·1923	0·0833
	K—	0·0294	0·0625	0·1071	0·0303
2nd	K	0·0882	0·0714	0·0714	0·0542
	K—	0·1500	0·1071	0·0909	0·0093
3rd	K	0·0556	0·0781	0·0741	0·0580
	K—	0	0·0536	0·0400	0·0326

TABLE IIC

Analysis of variance of rates of starch disappearance

	Degrees of freedom	Variance	Standard deviation	Z found	Z for $P=0·05$
Treatment	1	0·00475781	0·00475781	1·4645	0·77—0·72
Time	3	0·01260797	0·00420266	1·4025	0·62—0·55
Leaf	2	0·00767402	0·00383701	1·3069	0·67—0·61
Remainder	17	0·03194480	0·00187901		

(3) *Synthesis of starch in the leaves.*

The top, second, third and fourth leaves from sixty plants (again thirty normal and thirty potassium-starved) were placed in darkness with their petioles dipping into 5 per cent. glucose and at suitable periods of 48, 72 and 94 hours (determined by preliminary experiments) were tested for the amount of starch present. The leaves were left for 24 hours on distilled water to become free from starch before use, and no starch was present in the sample taken at the start, or in

controls throughout the experiment. The results given in Table IIIA are the averages calculated from the individual readings, each value being obtained from twelve to thirty readings. Analysis of the results (Table IIIC) shows that the formation of starch is on the average significantly more rapid in normal than in potassium-starved plants, and that there is a significant increase in the rate of formation with time. The early lag is in all probability associated with the initial penetration of the sugar solution into the synthesising cells.

TABLE IIIA

Starch present in the leaves

Leaf	Culture	After 48 hours	After 72 hours	After 94 hours
Top	K	1·9	2·1	2·2
	K—	1·7	1·25	1·6
2nd	K	0·9	2·5	3·4
	K—	0·4	2·3?	1·9
3rd	K	0·1	0·2	5·7
	K—	0·1	0·1	2·3
4th	K	0	0	3·0
	K—	0	0	0·5

TABLE IIIB

Rate of starch formation per hour

Leaf	Culture	0–48 hours	48–72 hours	72–94 hours
Top	K	0·0396	0·0083	0·0045
	K—	0·0354	−0·0188	0·0159
2nd	K	0·0188	0·0667	0·0409
	K—	0·0083	0·0791	−0·0182
3rd	K	0·0021	0·0042	0·2500
	K—	0·0021	0	0·1000
4th	K	0	0	0·1364
	K—	0	0	0·0227

TABLE IIIC

Analysis of variance of rates of starch formation

	Degrees of freedom	Variance	Standard deviation	Z found	Z for P=0·05
Treatment	1	0·02707063	0·02707063	3·0141	0·72−0·78
Time	2	0·01542710	0·00771355	1·7586	0·61−0·68
Leaf	3	0·00667162	0·00222387	0·5150	0·55−0·62
Remainder	17	0·02259265	0·00132898		

Invertase

Preliminary investigations were carried out with plants grown in January. The material was therefore in an extremely starved con-

dition, both light and temperature being subnormal, the normal plants being even more affected than those that were starved of potassium, i.e. paler in appearance and with smaller leaves, and fewer leaves per plant remaining green and unshrivelled. All the leaves of the normal plants were gathered together, and those of the starved plants were also treated as a single sample, no account being taken of the differences in age or position of the leaves from the apex downwards.

The results were as follows:

Culture	Activity per unit area	Activity per leaflet
With potassium	7·68	0·20
Without potassium	8·51	0·23

In the winter-grown cultures there appears to be very little difference between the invertase content of the potassium-deficient plants and the normal plants, but the starved leaves have a slightly higher activity than the normal.

On the other hand, three similar estimations carried out on summer cultures gave quite different results, which are summarised below:

Culture	Activity per 1 gm. dry wt.
With potassium	26·13
Without potassium	20·34

Since a seasonal fluctuation was apparently being shown, another set of cultures was tested in March. Relative to the summer plants these were of a poor growth, though not so starved as the January cultures; the individual leaves were larger and more succulent. Results:

Culture	Activity per 1 gm. dry wt.	Activity per leaflet
With potassium	4·06	0·54
Without potassium	3·88	0·56

The invertase activity of plants grown in March is therefore about the same under normal or potassium-starved conditions of culture. A detailed investigation of the distribution of invertase was carried out with plants grown in May and June.

Each group of cultures was divided into three sets, as nearly equivalent as possible, and the activity of the extract of each set was determined separately by means of three digests, from each of which three estimations were carried out. Thus in the final averages of the results every value was obtained from twenty-seven experimental

readings. Again the results are expressed in a number of different ways (see Table IV). The distribution of invertase is completely different from that of diastase and the graph (Fig. 2) shows a cross-over. In the normal plant the activity is highest in the top leaves, falling rapidly in the second and third leaves and then remaining uniformly low in the more mature leaves. Under potassium starva-

TABLE IV

Distribution of invertase

Leaf	Cultures	Per unit area	Per 1 gm. fresh wt.	Per plant	Per leaflet
Top	K	3·588	137·4	44·017	7·577
	K−	2·175	87·53	19·633	3·443
2nd	K	1·281	65·79	39·541	6·967
	K−	0·956	49·54	23·712	4·180
3rd	K	0·608	32·31	37·699	24·071
	K−	0·591	30·00	29·116	5·621
4th	K	0·337	15·52	24·071	5·321
	K−	0·312	14·13	16·363	3·542
5th	K	0·201	8·75	13·561	3·193
	K−	0·257	13·49	13·118	3·272
6th	K	0·208	9·55	13·071	3·601
	K−	0·315	15·63	14·191	4·439
7th	K	0·170	7·21	7·69	3·080
8th	K	0·074	2·50	2·00	0·959

tion the activity of the top and second leaves is lower than that of the normal leaves, but the activity increases again in the mature leaves and is then higher than that of the normal leaves. The seasonal effect noted above may be due to the shifting of the point at which the crossing-over occurs, for the relative activity of the normal and potassium-starved plants (if all the leaves are estimated together) will show the normal or deficient plants with a higher activity according as this point shifts towards the base of the plant or towards the apex.

Catalase

The plants used for the determinations had developed during June and July and had therefore made good growth.

A preliminary experiment gave irregular results, but this was found to be due to differences in activity of the enzyme from different leaflets of any given leaf. One leaflet had been selected at random from each leaf, since only a small quantity of leaf material was needed for a determination of catalase activity. For the results to be strictly comparable, however, either leaflets of a definite position should be

compared or the entire leaves tested. The second method was more reliable since the number of leaflets per leaf varied from nine to two,
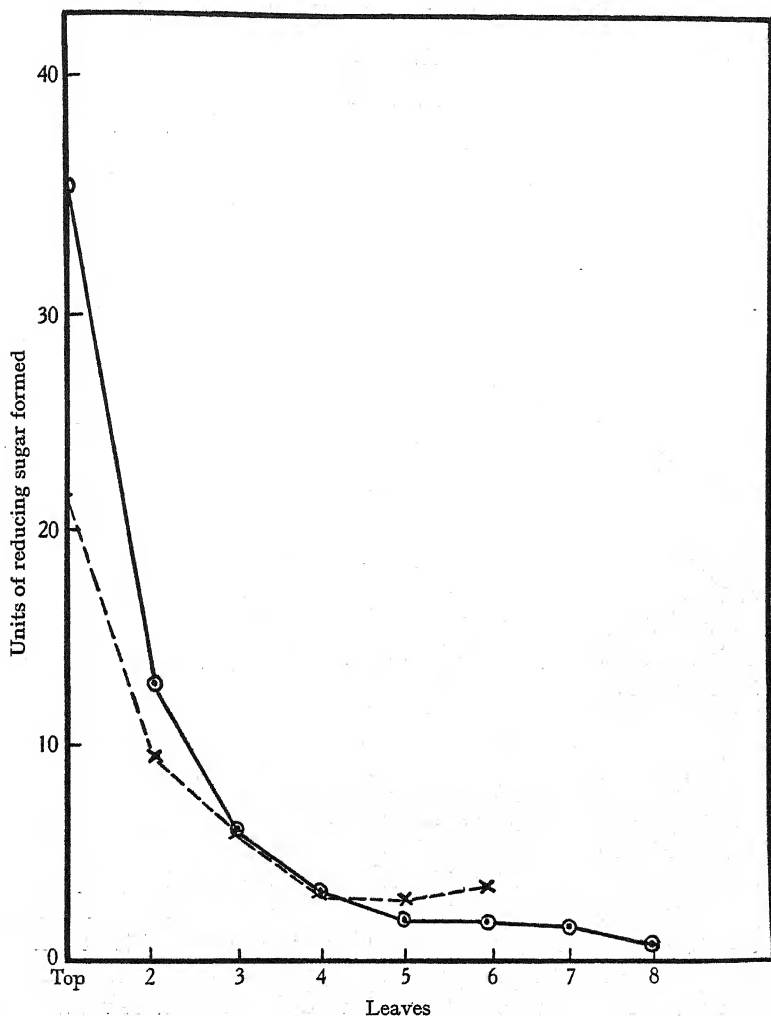

Fig. 2. Distribution of invertase in leaves, expressed as units of reducing sugar per 100 sq. cm. of leaf.

⊙— plants grown with complete culture solution.
×--- plants grown without potassium in the culture solution.

the number decreasing with increasing distance from the growing point.

A set of eighteen plants (nine normal and nine starved) was used for the investigation of catalase distribution, and twelve digests were set up for each leaf sample. The average results are given in Table V.

TABLE V

Distribution of catalase

Leaf	Culture	Oxygen per leaflet	Oxygen per unit area	Oxygen per 1 gm. fresh wt.
Top	K	15·46	4·841	273·1
	K—	6·75	2·252	129·0
2nd	K	44·69	7·767	404·2
	K—	36·03	6·624	351·2
3rd	K	14·23	1·702	91·6
	K—	16·57	2·240	118·4
4th	K	22·30	2·400	125·4
	K—	15·01	1·905	116·1
5th	K	20·38	2·065	111·3
	K—	10·25	1·046	55·2
6th	K	17·05	1·304	71·0
7th	K	15·62	0·910	48·6
8th	K	10·15	0·664	33·8
9th	K	4·52	0·318	18·7

The distribution of catalase (Fig. 3) is seen to be essentially similar to that of diastase, and is entirely in agreement with the results of other workers, thus lending some support to the suggestion that the activity of this enzyme parallels the general metabolic activity of the organism.

CONCLUSION

Although the results given in this paper show a certain agreement with those of other workers, it is impossible to make a strict comparison with the papers of Doby and Hibbard (1927), Hartt (1929), etc., owing to differences in material used and in methods of sampling and of enzyme estimation. The enzyme content of the leaves is shown in the present paper to be due to a complex interplay of position on the plant, season and manurial effects, and a method of sampling which will allow of comparison of leaves of different ages must be used.

Hartt, working with sugar-cane, gives results for the effect of potassium starvation on a number of different enzymes including diastase, invertase and catalase. The results for catalase are in agreement with the results described above, and the invertase content of the blades in June was decreased by withholding potassium. Diastatic activity was higher in potassium-starved than in normal plants in

April and June. These results were, however, obtained from single plants and on this account are of limited value. Englis and Lunt
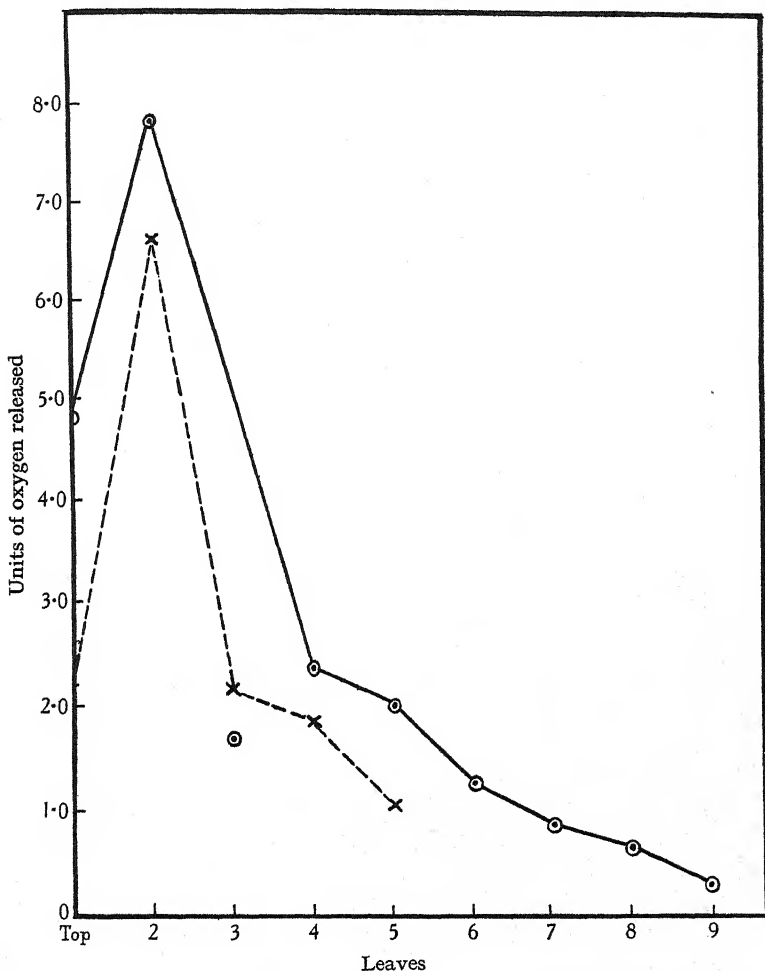

Fig. 3. Distribution of catalase in leaves expressed as units of oxygen released per 1 sq. cm. of leaf.

⊙— plants grown with complete culture solution.
×--- plants grown without potassium in the culture solution.

(1925) found that the application of potassium to soil cultures of *Tropaeolum* gave no change of diastatic activity. This may have been due to the soil initially containing potash in excess of the plant's needs.

The work of Doby and Hibbard is free from such objections, and the results of these workers show that the amount of diastase extracted from potassium deficient plants of sugar beet is greater than that from the normal plants. The extraction was from fresh material, however, and it is possible that complete extraction of the enzyme was more nearly obtained in the starved plants owing to a deficiency of combining substances, than in the normal plants. It is impossible to compare these results with the enzyme content of the bean recorded above, as the estimations carried out on sugar-beet were of starch disappearance, and those on bean were of sugar formation, and the effect of potassium starvation may be different for each reaction. Again, in estimating the enzyme content of the sugar-beet leaves, no account was taken of age or seasonal effects. The results given above for beans grown in winter show that the average difference between normal and starved plants is considerably less pronounced and in plants of a different habit this might even show a complete reversal. Similarly the intersection of the invertase graphs provides a possible means of bringing the bean results into line with those from sugar-beet, since a shifting of the point of crossing-over will cause considerable alteration in the "average" results.

It is not proposed to discuss the mechanism of this effect, since further work is in progress to this end, but it may be pointed out that the diastatic effect does not seem to depend upon a control of the amino-acid content.

SUMMARY

1. The effect of potassium starvation on the distribution of diastase, invertase and catalase is examined in bean plants grown in water culture.

2. For diastase the following rates were determined:

(a) rate of formation of sugar from Lintner's soluble starch by leaf extract;

(b) rate of disappearance of starch from darkened leaves;

(c) rate of formation of starch in leaves floated on glucose solutions in the dark.

The amino-acid content of the leaves was determined for comparison.

3. Invertase and catalase activities were determined in the usual way.

4. The results obtained are summarised as follows:

	Leaf					
	1	2	3	4	5	6
Amino-acid content	+	+	±	∓	−	−
Diastase:						
(a) Formation of sugar by extract	+	+	+	+	+	+
(b) Disappearance of starch from darkened leaves...	←————————— + —————————→					
(c) Formation of starch in darkened leaves	←————————— + —————————→					
Invertase	+	+	±	±	−	−
Catalase	+	+	−	+	+	.

Where the normal plants show a higher activity a + sign is used, and a − sign indicates the deficient plants with a higher activity. The arrows in (b) and (c) indicate an average result. Where there is little difference between the two sets of plants both signs are written.

ACKNOWLEDGMENT

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STUDIES IN THE MORPHOLOGY AND BIO-CHEMISTRY OF THE PINEAPPLE[1]

II. RESERVES IN THE SEEDS OF TWO GENERA OF THE BROMELIACEAE AND OF VARIOUS PINEAPPLE HYBRIDS

BY L. E. HOLMES, M.Sc.[2]

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(With 1 figure in the text)

FOLLOWING upon research on the anatomical structure of the seedlings and the morphology of the seed of the pineapple—the results of which were published in the *New Phytologist* (Thomas and Holmes(15))—interest was aroused in the food-storage products of the seed. Various hybrid forms of *Ananas* seed, obtained from Hawaii, were placed at my disposal by Dr E. N. Miles Thomas, who suggested that I should begin an investigation to try and discover if any differences could be detected in the food storage products of these seeds. As the different varieties of pineapple differ in the flavour of their fruits and therefore must differ slightly in the chemical composition of their fruits it was thought that small differences might also reveal themselves in the composition of the seeds. This has been found to be the case.

A study of the literature of the subject showed how complex is the problem of the composition of seeds—many factors, both environmental and hereditary, being involved in determining the products stored in any seed.

The influence of heredity on seed composition has been shown in several cases by comparing the proportion of compounds found in different varieties of the same plant when grown under the same external conditions. It has been shown in the case of wheat (Roberts(13)), maize (Guerrant(6)), peas (Guerrant(6)), soy-beans (Fellers(4), Lewkowitsch(10)), cotton seed (Schwartze and Alsberg(14)), etc., that different varieties of these plants when grown under the same conditions produce differing amounts of the various chemical products

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in their seeds. Selection experiments on maize at the Illinois Experimental Station and also by East and Jones[3] indicate that probably several Mendelian factors are involved for each type of compound found within the seed. By selection and self-pollination these workers changed the composition of maize grain to a considerable extent.

On the other hand, many external factors are known to cause quite large variations in the chemical constitution of seeds, e.g. water supply, available nitrogen and other soil conditions, time of planting, involving shortening or lengthening of the growing period, etc. In maize environmental factors may increase or decrease the protein content of the grain as much as 40 per cent. above or below the average. The different environmental factors cannot be separated wholly from each other—they are interdependent to a large extent. For example, the effect on the seed of differing amounts of water supplied to the plant depends partly upon the amount of nitrogen available in the soil (Jones, Colver and Fishburn[9]), also the effect of the nitrogen in the soil depends upon the period of growth at which it is available (Gericke[5]).
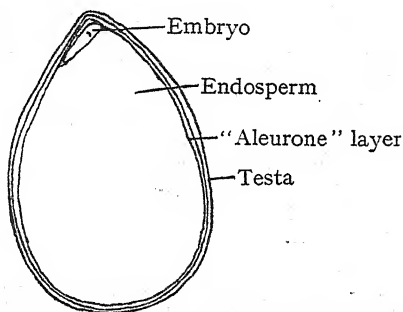

Embryo
Endosperm
"Aleurone" layer
Testa

Fig. 1. Longitudinal section of seed of *Ananas*.

Investigations into the chemical constitution of seeds have mainly been concerned with one particular compound in the seed, e.g. oil, protein or carbohydrate. As a rule, the seeds that have been investigated are those which contain some substance useful for human needs either as edible food or for medicinal or commercial purposes. Very few seeds have been fully investigated and those few are usually forms of great commercial value such as cereals. Where more detailed studies have been made into the types of oils (e.g. Hilditch[8], McNair[11], Lewkowitsch[10], etc.) or proteins (Csonka, Jones, Johns, see Onslow[12] Bibliography VII) found in seeds the great complexity of these compounds is shown, but there is also some indication of the

possibility of taxonomic relationships being revealed by comparing oils, etc., from closely related seeds.

The seed of the common variety of pineapple grown in Hawaii is of the simple Monocotyledonous type. It is an albuminous seed with a very minute embryo (Fig. 1). I found all the eleven varieties of *Ananas* listed below to contain the same three classes of compounds inside its seed, viz. carbohydrates, proteins and oil. Of these carbohydrate, in the form of starch, is present in far the greatest quantity composing the greater part of the reserve food of the endosperm of the seed. It is also present in a very small amount in the embryo. Protein is present in the endosperm in the form of minute granules between the starch grains and also to a small extent in the outer specialised layer of the endosperm commonly called the "aleurone" layer. The cells of the minute embryo, and also of the "aleurone" layer are filled mainly with drops of oil.

The seeds of *Bromelia pinguens* from Porto Rico (a genus closely related to *Ananas*) were obtained by Dr Thomas and handed to me for purposes of comparison. These seeds proved to be very similar to those of *Ananas*, the chief difference being one of size, as the *Bromelia* seeds are five or six times bigger than those of *Ananas* which measure only 5 mm. in their longest axis. *Bromelia* stores the same food products as *Ananas*, i.e. carbohydrates, protein and oil in the same parts of the seed.

The varieties upon which seed estimations were made, were the following:

A. *Ananas sativus*

(1) "Chance" Cayenne—self-fertilised seed from the commercial fields.

(2) Cayenne ♂ × Taboga ♀.

(3) (Cayenne × Wild Brazil) × Hilo Cayenne.

(4) Cayenne ♀ × F_1 hybrid ♂ (Ruby × Cayenne).

(5) Cayenne ♀ × Ruby ♂.

(6) Cayenne ♀ × Cayenne seedling E (E self-fertilised Cayenne).

(7) (Cayenne × Queen) F_2.

(8) (Cayenne × Smooth Guatamala) F_1.

(9) (Cayenne × Smooth Guatamala) F_2.

(10) Unknown cross—seed received from Porto Rico.

(11) Unknown variety (probably Cayenne)—original one obtained from Hawaii.

B. *Bromelia pinguens*

With the exception of Nos. (1) and (11) these are all hybrids. It is not known whether the hybrids are crosses between genetically pure varieties or not. The hybrids Nos. (2), (5), (6), (8) and (10) are first crosses; Nos. (7) and (9) are F_2 generations which means that the seed will be mixed; while Nos. (3) and (4) are back-crosses between Cayenne and a hybrid. All the hybrids contain "Cayenne" as one of the parent forms. Nos. (8) and (9) are the first and second generations respectively of the same cross.

METHODS

I have confined the investigations so far to determinations of the carbohydrate, nitrogen and water contents of the different seeds in the resting stage and, in one case only, that of the commercial "Cayenne", determinations were made also at various stages of germination. The following estimations were made on each lot of resting or germinated seed:

A. *Water content*

This was found by drying the seed at 95° C. on a water bath till the weight was constant.

B. *Nitrogen content*

The quantities of total nitrogen and also of soluble nitrogen were both estimated. The former was estimated by the micro-Kjeldahl method on 30 mg. of dried seed material, the estimations being carried out, as a rule, in triplicate. For the soluble nitrogen the dried seed was extracted with 60 per cent. alcohol—100 c.c. to every 200 mg. of dried seed. This precipitates the proteins. 10 c.c. portions of this extract were evaporated to dryness on a water bath and the nitrogen in the residue was estimated by the micro-Kjeldahl method in the same way as for the total nitrogen.

C. *Carbohydrates*

The quantities of total carbohydrates, total sugars and reducing sugars in the seed were estimated in each case in the terms of reducing sugars. The method of sugar estimation used was that of Willaman and Davis(16)—a modification of the picric acid method of estimating sugars in blood. In this method 1 c.c. of the solution, 2 c.c. of saturated picric acid solution, and 1 c.c. of 20 per cent.

sodium carbonate solution are heated together in a graduated test-tube in boiling water for 20–30 min. After cooling and diluting to 10 c.c. the solution is matched against a similar one containing a known percentage of glucose in a colorimeter. The total carbohydrates were found by treating 50 mg. of dried seed with 10 c.c. of $N/2$ sulphuric acid for three hours and estimating the sugars formed as above. The reducing sugars were estimated in portions of the 60 per cent. alcoholic extract used for the soluble nitrogen determinations. The total sugars were found from the same extract, but any non-reducing sugars present were transformed into reducing sugars by heating the extract and the picric acid solution together for 10 min. before adding the sodium carbonate solution and boiling again.

<center>RESULTS</center>

A. Resting seed

(1) *Water.* It was found that the resting stage of all the different varieties contained an appreciable amount of water—the amounts varying from 10 to 19 per cent. *Bromelia* seed has a similar water content to that of the *Ananas* seeds having actually 11·6 per cent.

(2) *Nitrogen.* The quantity of total nitrogen in the resting seeds is always in the close vicinity of 3 per cent.—varying only within 0·75 per cent. of this figure. There is only about 1·25 per cent. difference between the highest and lowest figures. *Bromelia* has the least percentage, but it is very close to the lowest of the *Ananas* percentages. The soluble nitrogen is always about a third of the total nitrogen, i.e. there is roughly 1 per cent. of soluble nitrogen in the dry seed. The F_2 generation of "Cayenne × Smooth Guatamala"(9) has an exceptionally low soluble nitrogen content and also a rather low total nitrogen content.

(3) *Carbohydrates.* The greater part of the carbohydrate present in the seed is starch and an estimate of the amount of starch present is gained by subtracting the amounts of total sugars from the amounts of total carbohydrates. The quantities of total carbohydrates in the different seeds vary over quite a large range, some having twice the percentage of others. *Bromelia* seed has a high carbohydrate content but within the same range as the *Ananas* seeds. Reducing sugars are present in considerable quantity in all the forms and the amounts vary from 10 to 17 per cent. The hybrid ("Cayenne × Taboga") which has one of the highest reducing sugar contents is the one with the smallest quantity of polysaccharide. Traces only of non-reducing

TABLE I

Composition of Ananas *and* Bromelia *seeds*

Seed	Water %	Nitrogen		Carbohydrate			
		Total in dry wt. %	Soluble in dry wt. %	Total %	Polysaccharide %	Reducing sugar %	Non-reducing sugar %
Ananas sativus							
(1) Chance Cayenne	13·63	3·17	1·07	36·16	25·76	10·4	0
(2) Cayenne × Taboga ♀	14·86	2·79	0·99	29·76	12·76	17·0	0·12
(3) Cayenne × Wild Brazil) × Hilo Cayenne	11·97	3·48	0·94	48·96	34·04	14·8	0
(4) Cayenne × F₁ hybrid ♂ (Ruby × Cayenne)	16·36	2·95	0·96	61·60	49·90	11·7	0
(5) Cayenne × Ruby ♂	10·58	2·93	0·96	65·92	51·12	14·8	0
(6) Cayenne × Cayenne seedling E	16·22	3·12	0·96	54·72	40·82	13·9	0
(7) (Cayenne × Queen) F₂	16·47	3·22	0·99	56·96	46·76	10·2	0·05
(8) (Cayenne × Smooth Guatamala) F₁	10·81	2·53	1·03	49·60	32·45	17·1	0
(9) (Cayenne × Smooth Guatamala) F₂	18·85	2·89	0·84	55·36	45·26	12·1	0
(10) Unknown cross from Porto Rico	13·14	2·71	1·16	48·12	34·72	10·4	0·22
(11) Unknown variety from Hawaii	17·64	2·84	1·07	45·12	32·40	12·5	0·03
Average for *Ananas* seeds	15·50	2·99	0·99	50·21	36·91	13·2	0·03
Bromelia pinguens	11·60	2·23	0·96	63·04	50·00	12·9	0·05

sugars could be detected in three of the *Ananas* hybrids and also in *Bromelia*, the remaining seeds giving no indication of them at all.

The results of the estimations on these seeds are contained in Table I.

This table shows that *Bromelia* seeds have a close similarity to *Ananas* seeds not only in type of compounds stored but also in their quantities. Compared with the average of the *Ananas* seeds *Bromelia* has a low water and total nitrogen content, a fairly low reducing sugar content, and a high content of polysaccharide.

These figures for the *Ananas* varieties show in particular the considerable range in carbohydrate content. In this respect the two forms which contain the highest percentage of carbohydrate are hybrids which obviously have a close relationship to each other. No. 5 with 65·9 per cent. is a hybrid of "Cayenne" and "Ruby," while No. 4 with 61·6 per cent. is a similar hybrid which has been back-crossed to the parent "Cayenne" form. This would seem to indicate that the parent "Ruby" possibly contains a factor for high carbohydrate content—higher than the "Cayenne" variety—and that while in the simple cross the Ruby character dominates, when there is a "double dose" of Cayenne the percentage tends to be lowered. It is noticeable too, that the simple hybrid No. 5 also contains a larger sugar content than the more complex No. 4. These two hybrid seeds are very similar in nitrogen contents—both total and soluble—being slightly below the average in each case. There is a marked difference in their water contents, No. 5 having a particularly low one, the lowest, in fact, of any.

The other two hybrids which are produced from the same strains are No. 8 and No. 9, being the first and second generations of the same cross—"Cayenne" and "Smooth Guatamala." These two do not show any close similarity but rather wide differences. The F_1 seed has decidedly larger soluble nitrogen and reducing sugar contents, but the F_2 seed has much the larger water content and somewhat bigger total nitrogen and carbohydrate content. It will be observed that the F_1 seed has an exceptionally high content of reducing sugar, while the F_2 seed has a smaller one but both are larger than the parent "Cayenne." Possibly "Guatamala" may have a factor for a much higher reducing sugar content than "Cayenne," and this factor is dominant in the F_1 generation, but in the F_2, where some of the seed will not contain "Guatamala," the percentage of these sugars is reduced over 25 per cent.

The No. 2—"Cayenne and Taboga"—hybrid shows figures which

are mostly well below the average with the exception of reducing sugar which is high.

Of the two unknown varieties the one from Porto Rico is characterised by a very high percentage of soluble nitrogen while its total nitrogen is rather low. In other respects it approaches the average. The other No. 11 from Hawaii also has a high soluble nitrogen content and a low total nitrogen content but its water content is rather high.

Compared with seeds of other plants worked out by other investigators *Ananas* and *Bromelia* seeds have a fairly average water content, one which is very similar to that of other Monocotyledons (with the exception of *Cocos* which is particularly high). The nitrogen content (which is equivalent to about 18–19 per cent. of protein) is very like most other seeds with a high carbohydrate content, e.g. *Amaranthus, Pisum*, etc. *The peculiarity of the seeds of these two genera, however, is their extremely high content of reducing sugar.* Most seeds, as far as recorded, have only a trace of these sugars, if any. The only other Monocotyledon which I have found recorded as having an appreciable amount of these reducing sugars in the seed is *Asparagus* which was investigated by Cake and Bartlett (2). *Acer saccharinum* (Anderson (1)) is peculiar in having a very high content of non-reducing sugars.

B. *Germinating seed and seedling*

During germination in the dark of the seed of the "Chance" Cayenne variety several changes in the storage contents occur. The total water content increases with the age of the seedling until after $11\frac{1}{2}$ weeks about four-fifths of the total weight of the seedling is due to water. During the first two weeks, during the imbibition of water before the seed begins to germinate, the water content more than doubles itself, but during the next two weeks when germination is beginning the increase is comparatively small, while during the following three weeks, when germination is well advanced and the endosperm is being used up, the water content again nearly doubles itself. Further increase during the next month results in a water content of nearly 80 per cent.

The percentage of total nitrogen in the germinating seedling fluctuates somewhat. There seems to be a decrease during the first month followed by an increase till the amount is greater than in the ungerminated seeds. The soluble nitrogen shows a similar decrease followed by an increase, but in this case the increase begins earlier

and is greater. The soluble nitrogen increases at the expense of the insoluble nitrogen. At first only a third of the total nitrogen is soluble, but after three months' growth in the dark more than half of it is soluble.

The amount of total carbohydrate in the seedling decreases with the increasing age of the seedling due to the starch in the endosperm being used up as germination proceeds. In one month the amount of polysaccharide has decreased to less than half of its original quantity, while at the end of three months practically no polysaccharide is left.

Reducing sugars increase in amount with increase in age of the seedling and non-reducing sugars, which could not be detected in the resting seed are present in minute amounts after the seedling is four weeks old. Table II shows the composition of the germinating seedling at various stages.

TABLE II

Composition of "Chance" Cayenne seedlings at various stages

Age of seedling	Water %	Nitrogen			Carbohydrate			
		Total %	Soluble %	Soluble N in total %	Total %	Polysaccharide %	Reducing sugar %	Non-reducing sugar %
Ungerminated	13·63	3·17	1·07	33·72	36·16	25·76	10·4	0·00
2 weeks old	34·95	3·03	0·98	32·31	—	—	13·5	0·00
4 weeks old	39·38	2·92	1·01	34·58	33·28	10·50	22·8	0·12
7 weeks old	65·77	3·09	1·26	40·82	32·64	11·14	19·5	0·17
11½ weeks old	79·85	3·25	1·68	51·64	23·68	0·41	23·1	0·17

Oil was not estimated quantitatively, but qualitative tests showed that it had disappeared in the seedling about the same time as the starch. This shows that the starch and the oil are the main reserve foods in the seeds that are used up as growth begins. Protein also shows a decrease in quantity but not a very large one. It is evidently partially transformed into other nitrogen compounds.

CONCLUSIONS

The differences in the quantities of the various products in the hybrid seeds of *Ananas* may be due to one or more of several causes. The actual conditions under which the different seeds were grown are not known but, as they were all produced under experimental conditions in Hawaii, it is probable that the conditions were somewhat

similar for each type of seed. Some of the differences found are too large to be wholly due to environmental conditions even if these varied to a large extent. For instance, the great variation in carbohydrate content between the "Cayenne × Taboga" hybrid (29·76 per cent.) and the "Cayenne × Ruby" hybrid (65·92 per cent.) must be due mainly to differences in variety, and as "Cayenne" (36·16 per cent.) is common to them both (and the probability is that "Cayenne" is a fairly pure variety as it is self-fertilised or reproduced vegetatively), presumably to original differences in the carbohydrate content of the parents "Taboga" and "Ruby." "Taboga" also appears to be characterised by a high sugar content which includes a trace of non-reducing sugar, while "Ruby" is more like "Cayenne" and has only an average sugar content none of which is reducing.

The one variety in which environmental conditions are most likely, and indeed must be different, is No. 10, an unknown cross received from Porto-Rico—where soil conditions, etc., would not be the same as in Hawaii. This variety is fairly average in its carbohydrate content but is peculiar in having an exceedingly low total nitrogen content and a very high soluble nitrogen content. As neither its conditions of growth nor its ancestry are known it is probable that both environmental conditions and heredity combined are responsible for these figures.

The *Bromelia* seed certainly shows a close similarity to *Ananas*. This is not unlike the similarity shown by two species of *Amaranthus* investigated by Harding and Egge[7] and by Woo[17]. More often, however, it is the type of fatty acid in the oil or of amino acid in the protein and not the quantities of oils or proteins which shows biochemically the taxonomic relationship between seeds.

SUMMARY

Two Bromeliaceous genera, *Ananas sativus* and *Bromelia pinguens*, have been investigated.

Eleven varieties of seeds of *Ananas*, mostly hybrids, showed a considerable range in composition—particularly in carbohydrate content—the main food store.

Bromelia seeds showed a close similarity to *Ananas* both in type and in relative quantities of compounds stored. All the varieties investigated differ from the majority of seeds in having a high reducing sugar content.

The seedling of one of the *Ananas* variety investigated at various stages of growth showed a gradual depletion of starch but a tendency to increase the nitrogen content.

The differences found in the composition of the different seeds are, for the most part, more likely to be due to hereditary than environmental influences.

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REVIEWS

The Woodlands and Marshlands of England. By H. A. WILCOX (Mrs G. S. Treleaven). University Press of Liverpool; Hodder and Stoughton, London, 1933. Pp. 55 with two large maps. 6s.

This book is the result of a serious attempt on the part of a professional geographer to establish and present an account of the distribution of woodland and marsh in England at two periods, viz., before the influence of man had become marked upon the vegetation (taken as approximately the Bronze Age), and about the end of the Mediaeval period. The results are given in the form of two maps on a scale of 1 : 1,000,000 with about 55 pages of written matter. The distribution of woodland and marsh in the earlier map is based on geological, topographic and climatic evidence, from which the nature of the vegetational cover has been deduced on general ecological grounds. The later map has been based upon actual maps of the countryside or upon clear written accounts indicating first-hand knowledge of the position of wood and marsh. Comparison between the two maps is extremely instructive: one of the most striking features is the large extent of deforestation which took place on large parts of the Liassic and Triassic areas of the Midlands and Lincolnshire, where apparently not the soils most readily cleared, but those of greatest agricultural value were exploited, leaving, even up to the present day the sparser woodlands of areas like Sherwood Forest upon the formations with poorer soils.

For the establishment of such points as these the book will be extremely useful, and there can be no doubt as to the desirability of making some such compilation as this, both as a background for archaeological investigation and as a record of the geographical and botanical history of this country. The task however is a stupendous one. The volume of reference to original botanical, geological, archaeological and historical works needed for either of these two maps, to say nothing of the need to have visited all parts of the country, places the task beyond the powers of any single person, even of the highest calibre.

The author has surmounted the difficulty by making extensive use of the more generalised text-books which deal with various aspects of her subject, but these naturally lag somewhat behind original investigation and tend to a simplification which though carefully qualified may be all the same dangerously misleading. Thus Clement Reid's splendid little book on "Submerged Forests" is the source of a great deal of the information in this book about our coastal peats, but since it was written the technique of pollen analytical treatment of peats has been developed and post-war archaeology has developed into more exact lines. Mrs Treleaven evidently accepts the view that earth movement ceased in this country, 3500 years ago, and suggests that the newest of the submerged forests cannot therefore be younger than this. From the archaeological investigation of Romney Marsh it is quite clear that there has been post-Roman, and even post-Norman subsidence; Roman remains occur in the Thames estuary at levels showing occupation many feet below the lowest level which would now be possible; Professor Swinnerton has actually shown the upper forest peat bed on the Lincolnshire coast to be of the Iron Age. These are but a fraction of the well authenticated data which bear upon a single point in the book. It is also becoming clear that there are not merely a lower and a "superior" peat bed upon the British coasts. In the North Sea and at various points along the east, south and western coasts, peats of Boreal age have been established. At lower Halstow, peat of Atlantic age appears associated with an industry of the Scandinavian Kitchen Midden type; along the Essex coast Hazzledine Warren has shown the lower coastal peat to overlie Bronze Age

settlement horizons of various periods, and from the submerged forests round the coasts Bronze Age objects are certainly the best known type of artefact. As we have said, Iron Age peats occur on the coast at Ingoldmells in Lincolnshire, and also in the Glastonbury levels. The peats at St German's near King's Lynn, Norfolk, included a bed containing beads of Roman or La Tène age, and this was covered by a bed of silty clay with abundant marine Foraminifera: above this bed again came yet another peat bed and more silty clay. Thus it will be seen how extremely complex is the problem of dating the coastal peats....It is past the point of simplification to terms of two beds, or cessation of movement in the Bronze Age. Indeed the recent paper on the Subsidence of London has shown that in the last sixty years our Ordnance Surveys have revealed a subsidence amounting to as much as two and a half feet in some parts of East Anglia.

The author is in similar difficulties over the status of individual species of British trees. Of the beech she says in one place "This tree is certainly not indigenous," and shortly after "it is quite likely that the beech was a constituent of the woods (of S.E. Britain) in the latter half of the Bronze Age." In fact the question is still *sub-judice*, but if the tree was indeed in Britain in the late Bronze Age there is no need to regard it as introduced, it would indeed coincide fairly well with the known period and rate of extension of beech forests across Europe and into the countries bordering the North Sea. Similarly with the pine. The author says it probably died out in Neolithic times, to be reintroduced in the eighteenth century, and she several times suggests that pine pollen indicates great age in a peat deposit. Certainly in the base of the Chat Moss peats the pine forests may be regarded as Boreal, but pine forests of much later date certainly occur elsewhere about the country. Thus the Iron Age peat of the Lincolnshire coast contains abundant pollen of the pine, and in the Cambridgeshire fenlands there are buried pine forests which are almost certainly post-Bronze Age. Again the problem is still unsettled as to the past history of the pine in this country, and demands much more investigation before generalisations can be made. It would be possible to say the same about the references to the past climate of this country. The general tendency has been to transfer to this island the sequence of climatic conditions established for the Continent, especially the Scandinavian scale of Blytt and Sernander. This is no doubt broadly adequate, but it really ought to be backed by a large mass of direct internal evidence of past climatic conditions within this country itself. This means still more specialist investigation. It is not desired to minimise the great amount of work which the author has put into her task, but it should be realised that the maps, especially the earlier one, is valid only in a general way and does represent close detailed analysis of recent original work, nor in the nature of the undertaking could this be expected; it is really a task for the co-operation of a large number of specialists over many years. The later map is based much more securely upon direct evidence, and its value is correspondingly higher.

The book is very well produced and, considering the size and quality of the maps, is very cheap at six shillings.

<div align="right">H. GODWIN</div>

Handbuch der Pflanzenanalyse. Herausgegeben von G. KLEIN. Dritter Band. Spezielle Analyse. Zweiter Teil. Julius Springer, Wien, 1932. RM. 162; gebunden RM. 168.

The massive proportions of this work become more obvious with the appearance of each successive volume. This, the third, is issued in two halves, and contains over 1600 pages, devoted to twenty articles from various pens. The net is spread so widely that not only does it include constituents of the living plant, but also "fossil plant materials," i.e. humus, peat, coals and oily shales.

The word analysis appearing in the title is also very liberally interpreted. Wherever any doubt may exist about the amount of description of compounds

necessary, it appears to have been settled on the side of generosity. Sections such as the "biological properties of saponins" and the "biological significance of lignification" seem to be definitely misplaced, but those who like to have everything within a uniform set of covers, and are able to indulge their tastes regardless of cost, will have good reason to be satisfied by this comprehensive work.

The articles in this volume deal with the following substances: wall materials (cellulose and all its allies), natural tannins, lichen acids, etherial oils, rubber, resins, glucosides with aliphatic and aromatic radicles, flavones and related pigments, anthocyanins, anthracene glucosides, cyanogenetic glucosides, indoxyl glucosides, leek and mustard oils, saponins, digitalis glucosides, glucosides of uncertain constitution, carotinoids of the higher plants, chlorophyll, algal pigments, fungal and bacterial pigments, and lesser known plant pigments. As in previous volumes, many of the articles are completed with lists by Wehmer and others of the distribution of the substances concerned. There is also an index covering 137 pages and 67 diagrams of apparatus, crystal forms, etc. The general form and presentation are of the same high standard as in the preceding volumes.

<div align="right">W. O. J.</div>

Liverworts of the Western Himalayas and the Panjab Plain. Part I. By SHIV RAM KASHYAP. Published by the University of the Panjab, Lahore, 1929. Pp. ii + 129, Plates I–XXV, 1 map. Part I, Supplement. By SHIV RAM KASHYAP. Published by the University of the Panjab, Lahore, 1932. Pp. 10, 1 text-fig. Part II. By SHIV RAM KASHYAP, assisted by RAM SARAN CHOPRA. Published by the University of the Panjab, Lahore, 1932. Pp. ii + 137, Plates I–XXXI.

It is now nearly twenty years since Prof. Kashyap first described (*New Phyt.* **13**, 206–226 and 308–323, 1914; **14**, 1–18, 1915) some of the remarkable endemic genera which make the Western Himalayas specially interesting to the hepaticologist. It is therefore very satisfactory that he has at last given us a full account of the hepaticae of the region. His book is the more welcome in that comprehensive works on the hepaticae of countries outside Europe are still very few and far between.

After a short introduction, ending with a glossary of terms used, there is a systematic account of all the families, genera and species found in the area. For certain species which are stated by Stephani and Gola to occur, but have not been found by the author, translations of the original descriptions are given. In the large genera *Lejeunea* and *Plagiochila*, the taxonomy of which is notoriously in a chaotic state, species believed to be new have not been named, but merely designated "A," "B," etc., pending revisions, an excellent plan which might well be copied. Full details are given as to habitat and distribution and there are often interesting critical or biological notes as well. There are clear and useful drawings of every species. In an appendix a brief account is given of the climate of certain frequently mentioned localities, together with lists of the species found there. Parts I and II overlap slightly as the Anacrogynae and Riellaceae are included in both.

The region dealt with in this book has an enormous range of altitude and climate. The rainfall for instance varies from 110 in. at Mussoorie to 3 in. at Leh. Though 166 species are recorded altogether, they are mainly concentrated in the wetter mountain region. The richest hepatic flora is found in the Outer Himalayas from 5000 to 8000 ft. and the number diminishes with the rainfall towards the inner ranges. In Western Tibet hepatics are said to be entirely absent.

The most striking feature of the Western Himalayan hepatic flora is its richness in Marchantiaceae, of which there are 20 genera and 41 species. There are several endemic genera, mostly monotypic, and many endemic species. This region is undoubtedly the "centre of gravity" of the family. The Jungermaniales are less characteristic.

The Panjab Plain, as might be expected from its climate, has a very poor hepatic flora, consisting almost entirely of thalloid forms. Interesting affinities with Mediterranean Europe are shown in the presence of species of *Petalophyllum* and *Riella*.

One point in this book calls for serious criticism. Though 12 new species are described in it not one of them is given a Latin diagnosis. The outlook for systematic botany is depressing if international conventions are set aside in this way.

As the author says in the preface, this book is rather a manual for the Indian field worker than a work of reference for the expert.
P. W. R.

SIXTH INTERNATIONAL BOTANICAL CONGRESS
AMSTERDAM, SEPTEMBER 2nd–7th, 1935

The Organising Committee of the Sixth International Botanical Congress has been asked by numerous correspondents to change the dates of this Congress; the Committee has now decided that the Congress will meet at Amsterdam (Holland)

SEPTEMBER 2nd–7th, 1935.

A first notice regarding this Congress has been sent out to many addresses; for additional copies please apply to the secretary, Dr M. J. Sirks, Wageningen, Holland.